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09/889874

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/GB00/00219	24 January 2000	22 January 1999

TITLE OF INVENTION
BIOLOGICAL CONTROL OF NEMATODES

APPLICANT(S) FOR DO/EO/US
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Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to promptly begin national examination procedures (35 U.S.C. 371(f)).
4. ☒ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)) (unsigned).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 16 below concern other documents or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

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I hereby certify under 37 CFR §1.10 that this correspondence is being deposited with the United States Postal Service as Express Mail Post Office to Addressee with sufficient postage on the date indicated below and is addressed to the Commissioner for Patents, Washington, D.C. 20231.

7-23-2001 Samantha Bell Samantha Bell

Date of Deposit Signature Typed Name of Person Signing

JC17 Rec'd PCT/PTO 23 JUL 2001

U.S. APPLICATION NO. (IF KNOWN) 09/889874	INTERNATIONAL APPLICATION NO. PCT/GB00/00219	ATTORNEY'S DOCKET NUMBER 13384-002001

17. ☒ The following fees are submitted:

Basic National Fee (37 CFR 1.492(a)(1)- (5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1000**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$860**

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO..................... **$710**

International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4).................. **$690**

International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4)........................... **$100**

				CALCULATIONS	PTO USE ONLY
			ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00	
Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$0.00	
Claims	Number Filed	Number Extra	Rate		
Total Claims	34 - 20 =	4	x **$18**	$72.00	
Independent Claims	3 - 3 =	0	x **$80**	$0.00	
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)			+ **$270**	$270.00	
			TOTAL OF ABOVE CALCULATIONS =	$1,202.00	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.				$0.00	
			SUBTOTAL =	$1,202.00	
Processing fee of **$130** for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f))				$0.00	
			TOTAL NATIONAL FEE =	$1,202.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31).**$40.00** per property +				$0.00	
			TOTAL FEES ENCLOSED =	$1,202.00	
				Amount to be refunded:	$
				Charged:	$

a. ☒ A check in the amount of $1,202.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 06-1050 in the amount of $0.00 to cover the above fees. A duplicate copy of this sheet is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 06-1050. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b) must be filed and granted to restore the application to pending status.

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14/PRTS

BIOLOGICAL CONTROL OF NEMATODES

TECHNICAL FIELD

The present invention relates to methods and materials for controlling nematodes.

PRIOR ART

Several thousand species of nematodes, sometimes called eel worms, are known. Numerous nematodes attack and parasitize humans and animals and cause disease. Additionally, several hundred species are known to feed on living plants. Certain of these are reviewed by Agrios in "Plant Pathology - 3rd Ed" Pub Academic Press Inc, see Chapter 15 therein.

Methods of controlling nematodes and their associated diseases include cultural practices; biological methods, e.g. use of resistant varieties; physical methods, e.g. heat; and use of chemical agents.

Patent application WO 92/19739 (Mycogen) relates to genes and gene fragments from *Bacillus thuringiensis* which have nematocidal activity. These generally encode crystal toxins from particular strains.

Patent application EP 0 303 426 (Mycogen) also relates to strains of *B. thuringiensis* which have nematocidal activity.

Patent application EP 0 171 381 (Monsanto) relates to particular soil bacteria which are capable of proliferating in an environment which is infested with

nematodes such as pseudomonads which colonise the surface of plant roots. The basis for the controlling activity appears to stem from glycosidase enzymes which are hypothesised to directly inhibit the nematodes.

Notwithstanding these disclosures, there is an ongoing requirement for materials which have nematocidal activity, for instance for use in crop protection or nematode-mediated disease control.

Patent application PCT/WO 99/22598 (University of Reading) published 14 May 1999 claims a biopesticide for the control of insect pests or plant parasitic nematodes or both, which comprises as an effective agent a species of bacteria which is a symbiont of an entomopathogenic nematode.

DISCLOSURE OF THE INVENTION

The present inventors have established that species of bacteria which in nature are associated symbiotically with entomopathogenic nematodes, can in fact be utilised to control nematodes, and in preferred forms of the invention, to kill them. The bacteria themselves can be employed, or nematode control agents can be used which are derived from such bacteria. In one aspect of the invention, the present invention employs bacteria which are engineered and thus not naturally occurring, or nematode control agents which are derived from natural or non-natural bacteria.

It has been reported that certain bacterial species such as *Xenorhabdus* and *Photorhabdus* can be used to control insects, see e.g. PCT/WO 98/08388 of MAFF, PCT/WO 97/17432 of WARF, and PCT/WO 99/42589 of Novartis. An effect against nematodes had not previously been demonstrated.

The symbiotic bacteria used in the present invention are isolatable from nematodes or the insects which the nematodes attack, and differ fundamentally in terms of life-style and activity from those soil bacteria such as *B. thuringiensis* or pseudomonads which have previously been suggested as being nematocidal.

Indeed, *prima facie*, it seems highly unlikely that nematode symbiotes might possess nematocidal activity. However, in the light of the present disclosure, a number of possible explanations for the observed activity can be tentatively proposed. Firstly, in order to protect a nutrient supply from a dead insect, the bacteria might produce anti-nematocides to prevent saprophytic nematodes gaining access. Alternatively, to become a symbiont, the bacterial strains may have once been pathogens of these nematodes and evolved towards a less hostile symbiotic relationship. The nematocidal activity may be an evolutionary throwback from the original pathogenic relationship, in which case it may be expected to be widely present amongst bacteria which have evolved in this way.

A first aspect of the present invention is the use of bacterial strains to control a target nematode, characterised in that in nature the bacterial strain is associated symbiotically with an entomopathogenic nematode.

As discussed in more detail below, the bacterial strains may be used in the methods of the present invention *per se*, or they may be used as a source of nematode control agent. The nematode control agent can be derived directly, or be prepared and utilised through recombinant DNA techniques, optionally via a host cell.

The target nematode will generally be different to the nematode with which the bacterial strain is found symbiotically in nature.

By means of the present invention employing bacteria or a nematode control agent, it becomes possible to control nematodes, in the sense of, to prevent or retard the effect that the nematode has on other organisms such as animals or more preferably plants, or to reduce the number of nematodes or nematode eggs in an area of interest, or to alleviate or cure a disease caused by nematodes. Control may be at the level of larval nematodes or nematode eggs, or may inhibit the motion, feeding or infectivity of adult nematodes. Nematocidal control may be employed to kill the nematode target. Such controlling activity can be assessed as shown in the Examples below.

PREFERRED EMBODIMENTS

The present invention provides a composition for the control of parasitic nematodes which comprises as an effective agent a species of bacteria which is a symbiont of an entomopathogenic nematode, or engineered bacteria having such activity, or a nematode control agent derived from natural or engineered bacteria.

Correspondingly, the present invention also provides a method of nematode control employing such a composition.

The bacterial species is typically of the genera *Xenorhabdus* or *Photorhabdus*, preferably the genus *Xenorhabdus*, for instance the species *Xenorhabdus bovienii*. Examples of particularly preferred bacteria include:

Xenorhabdus bovienii strain H31 deposited with NCIMB under accession number NCIMB 40985 on 20 January 1999;

Xenorhabdus bovienii strain I73 deposited with NCIMB under accession number NCIMB 40986 on 05 November 1998; and

Xenorhabdus strain C42 deposited with NCIMB under accession number NCIMB 41004 on 05 November 1998.

The nematode control agent can be a peptide derived from a symbiont of an entomopathogenic nematode or an engineered bacterium has functional activity against a nematode. The peptide nematode control agent can be produced from a nucleic acid derived from a symbiont of an entomopath nematode or an engineered bacterium and which encodes such a peptide. The peptide can be an oligopeptide or a polypeptide, notably a protein. In one version, the nematode control agent is a toxin with toxic activity against nematodes, but the nematode control agent can have other activity.

The nucleic acids of this invention can be employed in a method of producing a peptide comprising the step of causing or allowing the expression from a nucleic acid of this invention in a suitable host cell.

The nucleic acid can comprise a natural nucleotide sequence or a degeneratively equivalent sequence, and functional variants thereof. Variants include homologous variants encoding a peptide which is a nematode control agent, the nucleic acid having 70% or more DNA sequence identity and/or the peptide having 70% or more amino acid sequence identity. Especially preferred nucleic acids in p 13-1f and p 14-2f and variants thereof.

The present invention extends to nucleic acids having a sequence which is a derivative by way of addition, insertion, deletion or substitution of one or more nucleotides. The nucleic acid can contain longer expressed sequences such that the nematode control agent is expressed as a fusion protein.

Nucleic acids complementary to the nucleic acid encoding a nematode

control agent are also part of this invention.

Nucleic acids for use as a probe or primer having a nucleotide sequence of at least 15, 18, 21, 24 or 30 nucleotides, which sequence is present in, or complementary to, the nucleic acid encoding nematode control agent are further provided by this invention. In this respect, the invention extends to a method for identifying or cloning a nucleic acid for nematode control agent which method employs such a nucleic acid probe.

A method provided by this invention comprises the steps of:

(a) providing a preparation of nucleic acid from a bacterium,

(b) providing a probe,

(c) contacting nucleic acid in said preparation with said probe under conditions for hybridisation of probe to any said gene or homologue in said preparation, and,

(d) identifying said gene or homologue if present by its hybridisation with said probe.

The hybridisation conditions can be selected to allow the identification of sequences having 70% or more sequence identity with the probe.

In one embodiment, the method comprises use of two primers to amplify a nucleic acid encoding a nematode control agent, at least one of the primers having a conserved nucleotide sequence of at least 15 nucleotides.

A method is further made possible by this invention comprising the steps of:

(a) providing a preparation of nucleic acid from a bacterium,

(b) providing a pair of nucleic acid molecule primers, at least one of which is a primer,

(c) contacting nucleic acid in said preparation with said primers under

conditions for performance of PCR,

(d) performing PCR and determining the presence of absence of an amplified PCR product.

Additionally, the invention provides a recombinant vector comprising a nucleic acid of this invention. The vector is preferably capable of replicating in a suitable host such as *E. coli* or in *Xenorhabdus*. The vector can be a baculovirus. In a preferred feature, the nucleic acid is operably linked to a promoter or other regulatory element for transcription in a host cell. Vectors can further comprise any one or more of the following: a terminator sequence; a polyadenylation sequence; an enhancer sequence; a marker gene; a sequence encoding pesticidal material derived from *Bacillus thuringiensis*.

The vector can be a plant vector.

The vector of this invention can be introduced into a cell. Thus, a method for transforming a plant cell comprises the step of causing or allowing recombination between the vector and the plant cell genome to introduce the nucleic acid into the genome. The nucleic acid can be incorporated into chloroplast DNA, or into mitochondrial DNA.

Host cells comprising a vector are also part of this invention. The host cell can be a plant cell, which may be in a plant.

To this end, a method for producing a transgenic plant comprises the step of regenerating a plant from the transformed cell. In turn, plants of this invention extend to the progeny of such plants.

Examples of plants of this invention include crop species which can be

protected, notably maize, cotton, soya, rice, *Brassica* species, tomato, potato, sugar beet, barley, soybean, peanut, onion, rye, wheat, corn, banana, raspberry, bean. Decorative and other plants are also possible, e.g. rose.

A part of the propagule of the plants is also envisaged by this invention.

A method of influencing or affecting the toxicity of a cell such as a plant cell is provided where the method includes causing or allowing expression of a heterologous nucleic acid of this invention within the cells.

In a further aspect, the invention involves the use of a material selected from: an *X. bovienii* strain, a nematode control agent; a nucleic acid; a host cell; a plant; a peptide; or a composition of the invention, for the control of a pest, especially where the pest is a nematode and the material is used to control the nematode.

The present invention extends to control of helminthiasis in humans and other animals including domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. The nematodes to be controlled include *Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagosromuni, Chaberria, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterkis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris, Caenorhabditis* and *Parascaris*.

Target nematodes may be selected from the genera *Aphelenochoides, Anguina, Bursaphalenchus, Criconemella, Melodigyne, Ditylenchus, Globodera, Heliocotylenchus, Heterodera, Pratylenchus, Radopholus, Rotelynchus, Tylenchus, Trichodorus, Xiphenema,* and *Caenorhabditis*.

The compositions of this invention can be used in conjunction with *Bacillus*

thuringiensis or pesticidal materials derived therefrom.

In a further aspect, there is provided an antibody or fragment thereof, or a polypeptide comprising the antigen-binding domain of the antibody, capable of specifically binding a peptide of this invention.

Such an antibody or fragment can be obtained by immunising a mammal with the peptide, and is useful in a method of identifying and/or isolating a nematode control agent comprising the step of screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of the antibody of claim.

Some further aspects of preferred embodiments of the invention will now be discussed.

Bacterial strains

These can be derived from any entomopathogenic nematode. Preferred species are *Xenorhabdus* and *Photorhabdus*.

Potential sources of bacteria for use in the methods of the present invention may be identified by any preferred method. For instance, entomopathogenic nematodes can be isolated using an insect baiting technique such as that described by Bedding & Akhurst (1975) Nematologia 21: 215-227. Bacteria from nematodes identified as being pathogenic to the insect are isolated, cultured, and used as a source of nematocidal agent, e.g. by analogy with the methods used in the Examples below. Preferably *Xenorhabdus* or *Photorhabdus* species are used.

The preferred bacterial strains include ones which have the characteristics of

strain C42, I73 or H31 isolated by the present inventors. This *Xenorhabdus* strain has the following characteristics: rod shaped; motile; non-bio luminescent; blue on NBTA; produces antibiotics; resistant to ampicillin; forms circular colonies; has convex morphology; white colour.

This strain was presumptively identified as belonging to the genera *Xenorhabdus* since it was isolated from an insect killed by an entomopathogenic nematode and had the above characteristics. The strain has been deposited at the NCIMB (23 St Machar Drive, Aberdeen, AB24 3RY, Scotland) by the applicants under accession number NCIMB 41004 on 20 January 1999.

Further preferred strains of the present invention are two strains of *X. bovienii* designated H31 and I73 which have also been deposited under the terms of the Budapest Treaty at the NCIMB under the accession numbers NCIMB 40985 and 40986 respectively. These share characteristics of C42 in that they are rod-shaped; motile; non-bioluminescent; blue on NBTA; produce antibiotics; resistant to ampicillin; form circular colonies; and have convex morphology. The strains were identified as belonging to the species *X. bovienii* when compared to the *X. bovienii* type strain T228 using Restriction Analysis of the complete 16S rRNA gene and partial sequence analysis.

Target nematodes and diseases

The group of diseases described generally as helminthiasis is due to infection of an human or other animal host with parasitic worms known as helminths. Helminthiasis is a prevalent and serious economic problem in domesticated animals such as swine, sheep, horses, cattle, goats, dogs, cats and poultry. Among the helminths, the group of worms described as nematodes causes

widespread and often at times serious infection in various species of animals. The most common genera of nematodes infecting the animals referred to above are *Haemonchus*, *Trichostrongylus*, *Ostertagia*, *Nematodirus*, *Cooperia*, *Ascaris*, *Bunostomum*, *Oesophagosromuni*, *Chaberria*, *Trichuris*, *Strongylus*, *Trichonema*, *Dictyocaulus*, *Capillaria*, *Heterakis*, *Toxocara*, *Ascaridia*, *Oxyuris*, *Ancylostoma*, *Uncinaria*, *Toxascaris*, *Caenorhabditis* and *Parascaris*. Certain of these, such as *Nematodirus*, *Cooperia*, and *Oesophagostomum*, attack primarily the intestinal tract, while others, such as *Dictyocaulus* are found in the lungs. Still other parasites may be located in other tissues and organs of the body.

The bacteria and encoded toxins of the invention may be used as nematocides for the control of the nematodes and diseases discussed above. More preferably, however, they are used to control soil and plant parasitic nematodes. Particular crop species which can be protected include tomatoes, potatoes, sugar beet, barley, soybean, peanut, onion, rye, wheat, corn, banana, raspberry, beans. Decorative and other plants may also be treated e.g. rose.

Target nematodes may be selected from the genera Aphelenochoides, *Anguina*, *Bursaphalenchus*, *Criconemella*, *Melodoigyne*, *Ditylenchus*, *Globodera*, *Helicotylenchus*, *Heterodera*, *Pratylenchus*, *Radopholus*, *Rotelynchus*, *Tylenchus*, *Trichodorus*, *Xiphenema*. A further organism used in certain of the Examples below is *Caenorhabditis elegans*. Other target organisms and plants are discussed by Agrios in "Plant Pathology - 3rd Ed" Pub Academic Press Inc, see Chapter 15 therein.

As stated above, the target nematode will generally be different to that with which the bacterial strain is found in nature.

Methods of use of bacteria

The bacteria may be used in any appropriate method which brings them into contact with the target nematode, preferably such that they, or their products, are ingested or absorbed by the target nematode.

In particular, regarding plants, the bacteria may be formulated in a variety of ways so as to enhance stability. For instance they may be employed in admixture with substrates to protect the cells.

The mixture can be spread over, ploughed into or otherwise mixed with nematode infected or potentially infected soil.

Regarding animals, bacteria intended for enteric inoculation can be mixed with carrier material that is suitable for ingestion by the intended animals.

Isolation of agent

Nematode control agents of the present invention, which may be proteinaceous, or nucleic acids encoding them, may be isolated and/or purified from the C42, I73 or H31 bacteria described above, in substantially pure or homogeneous form, or free or substantially free of other materials from the bacterial strain of origin. Where used herein, the term "isolated" encompasses all of these possibilities.

Methods of purifying proteins from heterogenous mixtures are well known in the art, e.g. selective precipitation, proteolysis, ultrafiltration with known molecular weight cut-off filters, ion-exchange chromatography, gel filtration, etc. A particularly useful initial technique in this regard is ultracentrifugation. Further methods which are known to be suitable for

protein purification are disclosed in "Methods in Enzymology Vol 182 - Guide to Protein Purification" Ed. M P Deutscher, Pub. Academic Press Inc. Other references which outline techniques commonly used by those of ordinary skill in the art include "Protein Purification - principles and practice" Pub. Springer-Verlag, New York Inc (1982), and by Harris & Angal (1989) "Protein purification methods - a practical approach " Pub. O.U.P. UK.

Nematocidal activity may be assessed using a spread assay as discussed below.

The C42, I73 or H31 agent may be wholly or partially synthetic. In particular they may be recombinantly produced from nucleic acid sequences which are not found together in nature (do not run contiguously) but which have been ligated or otherwise combined artificially.

For instance, in the Examples below, nucleic acid encoding toxin(s) from I73 has been expressed in hosts cells using a vector system. Amino acid sequences of 38 different putative I73 toxin(s) are set out in sequence Annex I. These sequences are based on the nucleic acid sequence set out in Fig 2 ('chrim5'), a cosmid clone derived from I73 genomic DNA which conferred nematocidal activity upon *E. coli* cells into which it was introduced (i.e. significantly reduced nematode larval growth and development, and feeding). As detailed below, the entire amino acid sequence as set out in each case may not be required for nematocidal activity. In particular the portion up to the first Met in each sequence may be omitted, as may other portions which may not contribute to the nematocidal activity. Thus, not all the proteins or genes may be required for nematocidal activity, and usually there will be one or more principal proteins, though others may play supporting roles such as in enhancing the activity or encoding other nematocidal activities.

Thus isolated nematocidal agents comprising a polypeptide containing all, or a nematocidal fragment, of any of the depicted I73 sequences, form one aspect of the present invention. Preferred agents include those encoded by p14-2f and p13-1f. Other active variants of these sequences are also encompassed as described below.

Candidate agents for use in this invention to control nematodes extend to those from the bacteria described in PCT/WO 99/22598, as well as the insecticidal toxins and bacteria of PCT/WO 99/42589, PCT/WO 98/08388 and PCT/WO 97/17432, the disclosures of which are incorporated by reference.

Nucleic acids and variants

In one aspect of the present invention there is provided a nucleic acid molecule encoding a nematode control agent of the present invention, for example a toxin, as described above.

The nucleic acid may be derived from the sequence shown in Fig 2 or the complement (or degenerate equivalent) thereof. This sequence (cHRIM5) was itself derived from I73 and identified by its unexpected nematocidal activity. Regions of this sequence believed to correspond to genes of the present invention are described in Fig 3. Isolated nucleic acids comprising one or more of these regions which encode a nematocidal activity are particularly preferred.

In the light of the present disclosure, further nucleic acids of the present invention may be isolated using PCR or southern blotting or other techniques well known to those skilled in the art. This requires the use of two primers to specifically amplify target nucleic acid, so preferably two

nucleic acid molecules with sequences characteristic of the C42, H31 or most preferably an I73 toxin isolated as above are employed. Using RACE PCR, only one such primer may be needed (see "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, Academic Press, New York, (1990)).

Thus a method involving use of PCR in obtaining nucleic acid according to the present invention may include:
(a) providing a preparation of bacterial nucleic acid,
(b) providing a pair of nucleic acid molecule primers suitable for PCR, at least one of said primers being a primer based on a toxin from C42, H31 or I73,
(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,
(d) performing PCR and determining the presence or absence of an amplified PCR product. The presence of an amplified PCR product may indicate identification of a variant.

In a further aspect of the present invention there are disclosed nucleic acids which are variants of the C42, I73 or H31 toxin. A variant nucleic acid molecule shares homology (or identity) with all or part of the C42, H31, or most preferably I73 sequence discussed above.

Preferably sequence comparisons are made using FASTA and FASTP (see Pearson & Lipman, 1988. Methods in Enzymology 183: 63-98). Parameters are set, using the default matrix blosum62, as follows:
Gapopen (penalty for the first residue in a gap): -12 for proteins / -16 for DNA
Gapext (penalty for additional residues in a gap): -2 for proteins / -4 for DNA
KTUP word length: 2 for proteins / 6 for DNA.

Homology (similarity or identity) may be at the nucleotide sequence and/or encoded amino acid sequence level. Preferably, the nucleic acid and/or amino acid sequence shares at least about 70%, 75%, 80%, or 85% homology, most preferably at least about 90%, 95%, 96%, 97%, 98% or 99% homology.

Another method for assessing homology at the nucleic acid level is by hybridization screening. One common formula for calculating the stringency conditions required to achieve hybridisation between nucleic acid molecules of a specified sequence homology is shown in Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al, 1989, Cold Spring Harbor Laboratory Press:

$Tm = 81.5°C + 16.6\text{Log}[Na+] + 0.41$ (% G+C) - 0.63 (% formamide) - 600/#bp in duplex

As an illustration of the above formula, using [Na+] = [0.368] and 50-% formamide, with GC content of 42% and an average probe size of 200 bases, the Tm is 57°C. The Tm of a DNA duplex decreases by 1 - 1.5°C with every 1% decrease in homology. Thus, targets with greater than about 75% sequence identity would be observed using a hybridization temperature of 42°C. Such a sequence would be considered substantially homologous to the nucleic acid sequence of the present invention.

Variants of the present invention can be artificial nucleic acids. Alternatively they may be novel, naturally occurring, nucleic acids, isolatable using the information disclosed herein. Thus a variant may be a distinctive part or fragment (however produced) corresponding to a portion of the C42, I73 or H31 toxin. The fragments may encode particular functional parts of the agent or they may be used for probing for, or amplifying, sequences corresponding to C42, I73 or H31 toxin. Sequence variants which occur naturally may include homologs of the C42, I73 or H31 toxin from other

bacteria, including nematode-symbionts. Artificial variants (derivatives) may be prepared by those skilled in the art, for instance by site directed or random mutagenesis (i.e. nucleotide addition, deletion or substitution, optionally to lead to amino acid addition, deletion or substitution) or by direct synthesis. Preferably the variant nucleic acid is generated either directly or indirectly from an original nucleic acid encoding the C42, I73 or H31 toxin.

Changes may be desirable for a number of reasons, including introducing or removing the following features. Sites which are required for pre- or post-translation modification. Changes for codon usage preferences to enhance gene expression in different organisms. Leader or other targeting sequences (e.g. membrane or golgi locating sequences) may be added to the expressed protein to determine its location following expression. All of these may assist in efficiently cloning and expressing an active polypeptide in recombinant form. Other desirable mutation may be random or site directed mutagenesis in order to alter the activity (e.g. host specificity) or stability of the encoded polypeptide. Changes may be by way of conservative variation, i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Also included are active (nematocidal) variants having non-conservative substitutions.

Variant nucleic acids encompass all of these possibilities. When used in the context of polypeptides or proteins they indicate the encoded expression product of the variant nucleic acid i.e. variants of C42, I73 or H31 toxin e.g. variants of the I73 toxin sequences disclosed hereinafter.

Vectors and production of host cells

In one aspect of the present invention, the nucleic acid encoding the nematode control agent is provided in the form of a recombinant and preferably replicable vector.

Generally speaking, those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, Sambrook et al (1989) *supra*.

The permitted vectors include, *inter alia*, any plasmid, cosmid, phage or *Agrobacterium* binary vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable, and which can transform a prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally, e.g. an autonomous replicating plasmid with an origin of replication. Illustratively integration can occur into chloroplast DNA or into mitochondrial DNA.

Preferably the nucleic acid in the vector is under the control of, and operably linked to, an appropriate optionally inducible promoter or other regulatory elements for transcription in a host cell such as a microbial, e.g. bacterial, yeast, filamentous fungal or plant cell. The vector may be a bi-functional expression vector which functions in multiple hosts. In the case of genomic DNA, this may contain its own promoter or other regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell. The vectors and host cells into which they are introduced may be used to clone or otherwise

identify nucleic acids according to the invention.

The agent may be used as part of a viral vector which is itself pathogenic to nematodes.

Also of interest in the present context are nucleic acid constructs which operate as plant vectors. Specific procedures and vectors previously used with wide success upon plants are described by Guerineau and Mullineaux (1993) (Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148). Suitable vectors may include plant viral-derived vectors (see e.g. EP-A-194809). Suitable promoters which operate in plants include the Cauliflower Mosaic Virus 35S (CaMV 35S). Other examples are disclosed at page 120 of Lindsey & Jones (1989) "Plant Biotechnology in Agriculture" Pub. OU Press, Milton Keynes, UK.

Host cells

The toxin genes or gene fragments encoding the nematocidal agents of the subject invention may be introduced into a host cell, microbial, animal or plant. Expression of the toxin gene in the host cell results, directly or indirectly, in the intracellular production and maintenance of the nematocide.

Thus the present invention also provides methods comprising introduction of such a construct into a plant cell or a microbial cell and/or induction of expression of a construct within a cell, by application of a suitable stimulus e.g. an effective exogenous inducer.

Hosts may be used to assay the activity of particular sequences or

fragments. Hosts can also be used to generate quantities of toxin which can be employed in situ in suitable treated cells, or alternatively with suitable hosts, e.g., *Pseudomonas* viable microbes can be applied to the sites of nematodes where they will proliferate and where they or their products can be ingested by the nematodes. Higher organisms, preferably plants, can also be engineered with the toxin. The result in each case is a control of the nematodes. A host may be selected that can tolerate harsh environmental conditions and then grow when they improve, as illustrated by *Bacillus* species where the spores can exist under environmental extremes.

Characteristics of interest for use as a nematocide microcapsule i.e. a vehicle for the active agent include protective qualities for the nematocide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; leaf affinity; lack of mammalian toxicity; attractiveness to nematodes for ingestion; ease of killing and fixing without damage to the toxin; and the like.

Treated host cells

Where the cell is treated, the cell will usually be intact and be substantially proliferative form when treated, rather than in a spore form, although in some instances spores may be employed. Treatment of the microbial cell, e.g. a microbe containing the bacterial toxin gene or gene fragment, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin.

Viable hosts

Where the toxin gene or gene fragment is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is preferable that microorganism hosts are selected which are known to occupy the phytosphere (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the nematocide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots) of a wide variety of important crops. These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera *Pseudomonas*, *Erwinia*, *Serratia*, *Klebsiella*, *Xanthomonas*, *Streptomyces*, *Rhizobium*, *Rhodopseudomonas*, *Methylophilius*, *Agrobacterium* , *Acetobacter*, *Lactobacillus*, *Arthrobacter*, *Azotobacter*, *Leuconosroc*, and *Alcaligenes*; fungi, particularly yeast, e.g., genera *Saccharomyces*, *Cryprococcus*, *Kluyveromyces*, *Sporobolomyces*, *Rhodororula*, and *Aureobasidium*.

Plants as hosts

Nucleic acid encoding the nematocides of the present invention can be introduced into plant cells using any suitable technology, such as a disarmed Ti-plasmid vector carried by *Agrobacterium* exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882,

EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green et al. (1987) Plant Tissue and Cell Culture, Academic Press), electroporation (EP 290395, WO 8706614 Gelvin Debeyser) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman et al. Plant Cell Physiol. 29: 1353 (1984)), or the vortexing method (e.g. Kindle, PNAS U.S.A. 87: 1228 (1990d). Physical methods for the transformation of plant cells are reviewed in Oard, 1991, Biotech. Adv. 9: 1-11.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. It has also been used with filamentous fungi (see de Groot et al, 1998, Nature Biotechnology 16: 839-842).

Recently, there has also been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (see e.g. Hiei et al. (1994) The Plant Journal 6, 271-282)). Microprojectile bombardment, electroporation and direct DNA uptake are preferred where *Agrobacterium* alone is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, e.g. bombardment with *Agrobacterium* coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with *Agrobacterium* (EP-A-486233).

Generally speaking, following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewed in Vasil et al., Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications, Academic Press, 1984, and Weissbach and

Weissbach, Methods for Plant Molecular Biology, Academic Press, 1989.

The generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) Current Opinion in Biotechnology 5, 158-162.; Vasil, et al. (1992) Bio/Technology 10, 667-674; Vain et al., 1995, Biotechnology Advances 13 (4): 653-671; Vasil, 1996, Nature Biotechnology 14 page 702).

Combination nematocides

In further embodiments of the invention, bacteria associated with entomopathogenic nematodes or the toxins or products discussed above are used in conjunction with other nematocidal bacteria such as *B. thuringiensis* strains (e.g. from WO 92/19739) or pesticidal materials derived therefrom.

Materials for use in the present invention

The present invention also embraces materials for use in the methods above. These materials include the novel bacterial strains which are associated symbiotically with an entomopathogenic nematode and which are capable of controlling a target nematode. In particular the invention encompasses strain C42, I73 or H31 in isolated or substantially isolated form, or strains having the characteristics of C42, I73 or H31 (including nematocidal activity assessed as below).

Also embraced are compositions and formulations of these bacteria. These may comprise or consist of wettable powders, granules or dusts, mixed with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, methylcellulose, xanthan gum and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells,

peat moss, vermiculite, soil, seeds, other plant tissue and the like). The formulations may include spreader-sticker adjutants, stabilizing agents or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, or polymers.

Bacteria may be mixed with other material while in freeze-dried form, encapsulated in biodegradable or water-soluble material, or otherwise treated to prolong their viability or decrease their levels of metabolic activity during handling. If desired, the carrier material may contain assimilatable nutrient sources to support proliferation of the bacteria.

Also included are purified or substantially purified nematocidal agents (particularly proteinaceous agents) isolated or isolatable from the strains or host cells discussed above.

Thus the invention further discloses nematocidal compositions comprising one or more agents as described above. Such compositions preferably further comprise other nematocidal materials from other *Xenorhabdus* species or non-*Xenorhabdus* species. These other materials may be chosen such as to have complementary properties to the agents described above, or act synergistically with it.

Toxins of the invention for use with animals can be adapted to be administered orally in a unit dosage form such as a capsule, bolus or tablet, or as a liquid drench when used as an anthelmintic in mammals, and in the soil to control plant nematodes. The drench is normally a solution, suspension or dispersion of the active ingredient, usually in water, together with a suspending agent such as bentonite and a wetting agent or like

excipient. Generally, the drenches also contain an antifoaming agent. Drench formulations generally contain from about 0.001 to 0.5% by weight of the active compound. Preferred drench formulations may contain from 0.01 to 0.1% by weight, the capsules and boluses comprise the active ingredient admixed with a carrier vehicle such as starch, talc, magnesium stearate, or dicalcium phosphate. Where it is desired to administer the toxin compounds in a dry, solid unit dosage form, capsules, boluses or tablets containing the desired amount of active compound usually are employed. These dosage forms are prepared by intimately and uniformly mixing the active ingredient with suitable finely divided diluents, fillers, disintegrating agents and/or binders such as starch, lactose, talc, magnesium stearate, vegetable gums and the like. Such unit dosage formulations may be varied widely with respect to their total weight and content of the antiparasitic agent, depending upon the factors such as the type of host animal to be treated, the severity and type of infection and the weight of the host.

When the active compound is to be administered via an animal feedstuff, it is intimately dispersed in the feed or used as a top dressing or in the form of pellets which may then be added to the finished feed or, optionally, fed separately. Preferably, a carrier for feed administration is one that is, or may be, an ingredient of the animal ration. Suitable compositions include feed premixes or supplements in which the active ingredient is present in relatively large amounts and which are suitable for direct feeding to the animal or for addition to the feed either directly or after an intermediate dilution or blending step. Typical carriers or diluents suitable for such compositions include, for example, distillers' dried grains, corn meal, citrus meal, fermentation residues, ground oyster shells, wheat shorts, molasses solubles, corn cob meal, edible bean mill feed, soya grits, crushed limestone and the like.

Alternatively, the antiparasitic compounds may be administered to animals parenterally, for example, by intraluminal, intramuscular, intratracheal, or subcutaneous injection, in which event the active ingredient is dissolved or dispersed in a liquid carrier vehicle. For parenteral administration, the active material is suitably admixed with an acceptable vehicle, preferably of the vegetable oil variety, such as peanut oil, cotton seed oil and the like. Other parenteral vehicles, such as organic preparations using solketal, glycerol, formal and aqueous parenteral formulations, are also used. The active compound or compounds are dissolved or suspended in the parenteral formulation for administration; such formulations generally contain from 0.005 to 5% by weight of the active compound.

Further aspects of the invention include nucleic acids, vectors and host cells containing a heterologous construct according to the present invention, especially a plant or a microbial cell.

Such microbial cells may be treated as described in the methods above. Examples of chemical reagents are halogenating agents. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-invectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Bouin's fixative and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. The method of inactivation or killing retains at least a substantial portion of the bio-availability or bioactivity of the nematode control agent.

In all of the compositions discussed above, the nematocide concentration may vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The nematocide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the nematocide while the liquid formulations will generally be from about 16% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^{10} cells/mg, more preferably 10^7 to about 10^9 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare. The formulations can be applied to the environment of the nematodes, e.g., plants, soil or water, by spraying, dusting, sprinkling, or the like.

In addition to the above the invention includes plant cells which have been transformed with the genes of the present invention, and plants which include such plant cells.

EXAMPLES OF THE INVENTION

The invention will now be further described with reference to the following non-limiting Figures and Examples. Other embodiments of the invention will occur to those skilled in the art in the light of these.

FIGURES

Fig 1 shows the cHRIM5 cosmid vector and subclones used for sequencing, as described in Example 6.

Fig 2 shows the sequence of cHRIM5 (1-37544 bps).

Fig 3 shows the position and orientation of ORFs in the cHRIM5 sequence.

Fig 4 shows deletions of cHRIM5 tested for nematocidal activity.

Fig 5 illustrates cloning of nematocidal activity in PLEX.

Example 1 - Source of strains C42, I73 and H31

Strain C42 was obtained using an insect entrapment method. Insects which were killed on the surface of a soil sample were observed under a microscope at high magnification. Any that contained high numbers of bacteria and not fungal hyphae were presumed to have been killed by insect parasitic nematodes. The identified presence of nematodes also aids this identification step, but it is not essential. These samples were plated on to NBTA media (see Poinar & Thomas, 1984 Nematodes p238-280 in "Laboratory guide to insect pathogens and parasites" Eds. Poiner & Thomas, Pub. Plenum Press, New York). Any colonies that developed that had characteristic features (e.g. morphology, size, colour) of *Xenorhabdus* or *Photorhabdus* strains were selected. Non-luminescent colonies were presumptively identified as *Xenorhabdus*. The identity of those having nematocidal activity as assessed in Example 3, is further confirmed using 16s rRNA sequence data (see Brunel et al 1997, Applied and Environmental Microbiology 63,2: 574-580).

I73 and H31 strains were obtained in a similar way to strain C42 but they were identified as belonging to the species *X. bovienii* when compared to the *X. bovienii* type strain T228 using Restriction Analysis of the complete 16S

rRNA gene (see Brunel et al. 1997 Applied and Environmental Microbiology: 574-580), and partial 16s ribosomal RNA sequence analysis.

Example 2 - Cell growth and preservation

Subcultures of the *Xenorhabdus* species C42, I73 and H31 were used to inoculate three 9 cm diameter petri dishes containing L agar (10g tryptone, 5 g Yeast Extract, 5 g NaCl and 15 g agar per lt). Plates were incubated for 48 hrs at 26°C and the resulting growth harvested by scraping off bacterial cells and thoroughly resuspending in 40 mls of 5% w/v lactose. The cells were washed once by centrifugation (5000 x *g* for 10 mins), resuspended in 10 mls of 5% w/v lactose, dispensed into 1 ml aliquots and freeze dried (-60°C for 48 hrs) for medium term storage at 2°C. Other stocks were re-suspended in nutrient broth containing 10% w/v glycerol (Protect) and frozen at -70°C.

Example 3- Activity of cells against *Caenorhabditis elegans*

The bioassays were performed by allowing *C. elegans* to feed on live bacterial cell suspensions spread over the surface of Luria broth agar (Luria broth containing 1.2%w/v agar) in segmented square petri dishes (2.0 x 2.0 cm per test well). A minimum of three test wells, each containing 50-100 nematodes were used for each test. Mortalities were recorded after 3 days at 18°C.

C. elegans was cultured on *Escherichia coli* at 18°C on 9 cm diameter LB agar plates. Once the nematodes had colonised the complete plate they were re-subbed on a fresh plate to maintain stocks and the remainder re-suspended in 40 ml LB. The tube was allowed to stand for 15 min and the nematodes settled to the bottom. The concentrated nematodes were removed using a

sterile pipette and placed in 40 mls of fresh LB. The process was repeated 5 more times to wash the nematodes away from the *E. coli* cells. The nematodes were then diluted so that approximately 50 nematodes were present in 50 µl of LB.

The *Xenorhabdus* cells used were cultured in LB at 30°C/100 rpm for 24 hours and 50 µl spread on to the surface of each test well. The control *E. coli* cells were treated in a similar way but incubated at 37°C for growth. After application the wells were air dried for 30 min, and 50 µl of the nematode suspension placed in each well. Again the wells were air dried for 30 min. Plates were incubated at 18°C with 80% relative humidity for 3 days.

Xenorhabdus spp. C42, H31 and I73 gave 95% mortality, as compared with no significant effect for certain other *Xenorhabdus* bacterial strains and *E. coli*. Thus these results clearly show that cells from *Xenorhabdus* C42, H31 and I73 are an effective nematocide.

Example 4 - Cloning of nematode active gene from I73

Total DNA was isolated from I73 using a Quiagen genomic DNA purification kit (cat no. 10243). To isolate DNA, cells were grown in Luria broth (10g tryptone, 5g yeast and 5g NaCl per lt) at 26°C with shaking at 200 rpm to an optical density of 1.5 A600. Cells were harvested by centrifugation at 4000 x *g* and the DNA isolated using Quiagen 100/G tips, as per manufacturer's instructions. The purified DNA was stored at -20°C in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

To obtain a representative I73 library, total DNA was partially digested with *Sau*3A. Approximately 25 µg of DNA was incubated at 37°C with 0.25 units

of enzyme. At intervals of 5, 15, 30, 45 and 60 minutes, samples were removed and heated at 65°C for 10 minutes. To determine the size of the resulting DNA fragments, the samples were separated on a 0.5% (w/v) agarose gel. The samples containing a dominant DNA fragment size of between 30 and 50 Kb were combined and treated with shrimp alkaline phosphatase (Boehringer) for 20 minutes at 37°C. The DNA was ligated into the *Bam*HI site of the Stratagene cosmid vector Supercos1 (scos) and packaged into the *Escherichia coli* strain XL Blue 1, using a Gigapack II packaging kit (Stratagene) following the manufacturer's instructions.

To identify individual cosmid clones with activity to *C. elegans*, single colonies were grown in individual wells of segmented square petri dishes on Luria agar, containing 50 µg/ml ampicillin at 30°C for 24 hours. To each well, approximately 50, mainly L4 and adult *C. elegans* larvae were added in 50 µl of Luria broth. The dishes were incubated at 18°C and examined after 6 days for nematode development.

A total of 600 clones were examined and one coded cHRIM5 was found, which caused significant reduction in larval numbers, with no live L4 and adult larvae observed compared to on average, greater than 40 in all other clones tested.

Example 5 - Activity of cHRIM5, C42, H31 and I73 against *C. elegans*

Clone cHRIM5 was grown in 50 mls LB containing 50 µg of ampicillin per ml at 30°C/200 rpm for 40 hours. C42, H31 and I73 were grown in 50 mls LB at 26°C for 48 hours/200 rpm. Cultures were centrifuged at 4000 x *g* for 10 minutes, washed once and resuspended in 5 mls of PBS (0.05 mM phosphate buffer, 0.125M NaCl). To determine activity, 300 µl of cells were added in triplicate, to 1.2 ml of PBS containing 25, mainly L4 and adult *C.*

elegans larvae in multi well square dishes. As a control, an equivalent amount of XL 1 Blue *E. coli* cells containing Supercos 1 were used to determine nematode survival. The assays were incubated at 18 °C for 7 days before approximate nematode counts and observations were made.

Activity of cells on *C. elegans*

Cell line	No. and size of larvae/square	Cell turbidity
XL 1 Blue/Supercos 1	>100 (all stages)	Clear
XL 1 Blue/cHRIM5	<20(mainly small, L1,2 &3)	Cloudy
C42	<10	Cloudy
H31	<10	Cloudy
I73	<10	Cloudy

Thus cHRIM5, C42, H31 and I73 all gave a reduction in nematode numbers, and in particular cHRIM5 cells significantly reduced larval growth and development. All four strains caused a reduction in feeding (as indicated by the cloudy cell suspensions).

Example 6 - DNA and protein sequences

Plasmid and cosmid DNA for cloning was prepared using the QIAGEN midi system (tip 100, cat. No 12143). Cells were grown in Luria broth (Merck) at 37°C with shaking at 200 rpm for 18 hours. Cells were harvested by centrifugation at 6,000 x *g* and the DNA isolated as per manufacturers instructions. Restriction digestion (Roche, Life Technologies), dephosphorylation (Roche) and ligation (Life Technologies) were carried out using manufacturer's recommended conditions and as outlined by Sambrook et al. Transformation was accomplished using electrocompetant cells and a

BIO-RAD Gene pulser set at 12.5V cm^{-2}. Two µl of DNA was used to electroporate 80 µl of early log phase *E. coli* DH5 alpha cells washed 3 times in sterile water (centrifugation at 6000 x g for 5 mins) and resuspended in 1/100th the original volume in 10% (v/v) glycerol. Luria agar containing either kanamycin or ampicillin at 50 µg ml^{-1} were used to select clones where appropriate.

DNA sequence analysis of cHRIM5 was completed by sequencing a number of sub clones and primer walking, see figure 1 for the supercos vector, where the numbers are kBp. The sub clones used are as follows:

code	cHRIM5 treatment	vector used or remaining
A-380	*Hind*III digestion and self-ligation	deleted scos
B-387	*Bam*HI digestion and self-ligation	pUC 19-*Bam*H1 digestion
C-381	*Sal*I-*Bam*HI digestion	scos
E-391	*Sal*I-*Bam*HI digestion	pUC 19-*Sal*I *Bam*H1 digestion
F-392	*Sal*I-*Bam*HI digestion	pUC 19-*Sal*I *Bam*H1 digestion

Sub clone A-380 was constructed by digesting cHRIM5 DNA with the restriction enzyme *Hind*III and re-ligating fragments, this clone contains a deletion of the insert and scos cosmid DNA as the vector. Sub clone B-387 is a *Bam*HI digestion of cHRIM5 cloned into the plasmid pUC19 also cut with *Bam*HI and dephosphorylated. Sub clone C-381 was obtained by digesting CHRIM5 DNA with *Bam*HI and re-ligating the fragments, this clone contains the scos cosmid as the vector. Clones E-391 and F-392 were obtained by cutting cHRIM5 DNA with *Sal*I and *Bam*HI and ligating these fragments into the vector pUC19 also cut with these enzymes.

Sequencing was conducted using the artificial transposon AT2 (supplied by Perkin-Elmer-Applied Biosystems, Primer Island Transposition kit, cat No.

403015) using the cosmid cHRIM5 and all sub-clones as target DNA. One µg of cHRIM5 DNA was incubated with the transposon AT2 for 1 hour at 30°C in a final volume of 20 µl. After incubation the reaction was stopped by adding 5 µl of 0.25M EDTA, 1% (w/v) SDS, and heat treatment at 65°C for 30 mins. The DNA was desalted by dialysis against water. One µl of the reaction mix was used to electroporate 80 µl of early log phase *E. coii* DH5 alpha cells. Colonies were selected on LB media containing 50 µg/ml trimethoprim. Once inserted the transposon mutants were used to provide a range of positions of primer sites at random intervals throughout the clones. The two primers PI+ and PI- near the end of the transposon were used to generate sequence data. In addition standard primers for the pUC19 and scos vectors were used to generate sequence data at the ends of each clone. DNA for sequencing was prepared using the QIAGEN ion exchange media (qiawell8, cat. No. 17122). Clones were grown in 1 ml of Luria broth containing trimethoprin (50 µg ml^{-1}) for 18 hours. Cells were centrifuged at 13,000 x *g* for 5 mins and resuspended in 350 µl of buffer P1. After 5 mins 350 µl of buffer P2 was added and the samples incubated for 5 mins at room temperature. To this 350 µl of buffer P3 was added and the samples left on ice for 15 mins. After centrifugation at 13,000 x *g* for 15 mins the samples were loaded on the Qiagen column under vacuum, and washed with buffer QC. DNA was eluted with buffer QF (500 µl) at 50°C and isopropanol precipitated (0.8 vol). After centrifugation at 13,000 x *g* for 30 min, DNA was washed with 70% (v/v) ethanol and air dried for 10 mins. The final pellet was resuspended in 10 µl of water. Cycle sequencing reactions using the Perkin-Elmer Applied Biosystems division Big Dye reaction kit (cat No. 4303149) were prepared using standard conditions for plasmid and cosmid sequencing. Samples were analysed on ABI Automated Sequencers. DNA sequences were assembled using the DNA* software. The complete sequence of cHRIM5 was obtained by primer walking to join the final DNA contigs together. The final sequence of cHRIM5ed2 is shown

in Figure 2. Analysis of the DNA using the software Clone indicated a number of ORF illustrated in Figure 3 and 4. Corresponding protein sequences are also presented at Annex I.

Example 7 - Fragments that encode nematocidal activity

To identify smaller fragments that encoded nematocidal activity, a series of sub-cloning experiments were performed using *E. coli* DH5 alpha. Qiagen midi and miniprep methods, restriction and ligations were used as for previous examples. Nematicidal activity of all constructs was determined as described in Example 4. In Figure 4, we show the deletions of cHRIM5 tested for nematicidal activity. Restriction sites and genes are indicated. Size in base pairs indicated on the map line. A cHRIM5, B cHRIM6, C cHRIM7, D cHRIM8, E cHRIM8, F cHRIM10, G *Nde*I deletion of cHRIM8, H Approximate positions (arrows) of three AT2 transposon insertions (tn58, tn26, tn43) in cHRIM9.

The cosmid cHRIM5 (figure 4A) was digested with the enzyme *Sal*I and religated. The resulting sub clone cHRIM6, illustrated in Figure 4B showed nematicidal activity. cHRIM6 was digested with the enzyme *Sma*I and religated, producing sub-clone cHRIM7 (Figure 4C). CHRIM7 was digested with *Bgl*II and the kanamycin resistance gene block (*nptII*, Pharmacia) cut with *Bam*HI was ligated into it. After selection on LB containing kanamycin (50μg ml^{-1}) and ampicillin (50μg ml^{-1}) the clone was digested with *Sal*I and religated, in effect creating a deletion from the *Sal*I site to the *Bgl*II site of cHRIM6 to generate CHRIM8 (figure 4D). By cutting cHRIM8 with *Nru*I a further deletion was made to create cHRIM10 (figure 4F). All the above clones maintained nematicidal activity.

Deletion of cHRIM8 with *Nde*I, removed a portion of the p14-2f gene (figure

4G), this reduced nematicidal activity. This indicates that the p14-2f gene or protein are important for nematocidal activity. Transposon mutagenesis of cHRIM9 (a clone very similar to cHRIM7 but deleted with *Nar*I rather than *Sma*I) with the artificial transposon AT2 (Perkin Elmer Applied Biosystems) resulted in a number of inserts within this clone (figure 4H). Insert cHRIM9-tn43 was restriction mapped to an approximate position of bp 20,700 (on cHRIM5) within the p20-9r gene, this mutant retained nematicidal activity. This indicates that this gene is not essential for activity. Insert cHRIM9-tn58 mapped to an approximate position of bp 13,400 (on cHRIM5), within the p13-1f gene, nematicidal activity was reduced. This indicates that this gene, region of DNA or the blocking effect of the transposon in this position is important for activity. Insert cHRIM9-tn26 was restriction mapped to approximate position of bp 15,000 (on cHRIM5) within the p14-2f gene, nematocidal activity was reduced. This indicates that this gene, region of DNA or the blocking effect of the transposon in this position is important for activity.

Clone cHRIM6-tn43 was digested with *Bgl*II and *Not*I and cloned into the vector PLEX (Invitrogen cat. No. K450-01) cut with *Bam*HI and *Not*I. The *E. coli* strain used was GI742 supplied by Invitrogen. The resulting plasmid insert (PLEX-*Bgl*II/tn43, Figure 5) places the p14-2f and p13-1f genes under the control of the bacteriophage Lambda P_L promoter. Figure 5 illustrates the cloning of DNA encoding nematocidal activity in the expression vector PLEX, where: A, plasmid clone; B, insert and gene locations; Tpr, trimethoprim resistance; Apr, ampicillin resistance; P_L, bacteriophage lambda PL promoter; *, plasmid joins to form a circular molecule; **, incomplete genes. Selection of colonies on RMG media (described in the Invitrogen manual) containing ampicillin (50 μg ml^{-1}) and trimethoprim (50 μg ml^{-1}) prevents expression from the P_L promoter. Colonies were then cultured on LB containing Trimethoprim (50μg ml^{-1}) in 2.0 cm^2 wells for

nematocidal tests. The clone was active. This indicates that genes within this fragment have nematocidal activity. The clone PLEX-*Bgl*II/tn43 was digested with *Cla*I and religated, this resulted in a deletion of part of the p13-1f gene, this clone had reduced nematocidal activity indicating the importance of this gene.

All these results indicate that the genes and gene products of p13-1f and p14-2f are important for nematocidal activity. Other smaller genes within the *Bgl*II to *Nru*I sites of cHRIM10 and PLEX-*Bgl*II/tn43 may also be essential. In addition genes outside this region within the remaining cosmid clone (cHRIM5) may also encode products with nematocidal activity, or may enhance the nematocidal activity of genes in the smaller region (*Bgl*II-*Nru*I of cHRIM10 and PLEX-*Bgl*II/tn43).

Example 8 - Field trials

Activity of strains selected in accordance with the above methods, or from depositary institutions which include bacteria which in nature are associated symbiotically with entomopathogenic nematodes, may be further assessed in field trials as follows.

Symbiotic bacteria in the absence of their nematode host can be inoculated into one or more portions of a field which is infested with nematodes, or into containers containing unsterilised soil from such a field. The bacteria can be inoculated onto the roots of plants, or into seeds. Periodically treated and untreated areas or containers can be assayed for nematode larva, egg, or cyst counts and for the presence of the inoculated bacteria by methods well known to those skilled in the art. A reduction in the number of nematode counts in areas in which the symbiote bacteria are present indicates control of the nematodes otherwise found in the untreated areas or samples.

Annex I - amino acid sequences

SEQ ID NO:1

P0-0f

ISWFATGIPTVDALLAEEFWHGDKQAFPPFTCRFTHFDPDKEQDVTLVPSTEEAYWLHRA
LQGQPLHSEVYGDDGTAQAGIPYTVMDSRPQVRLLTGLPGNSPTVWPSVIEQRTWQYERI
ADDPQCHQQVVLNSDRYGFPRETVDIAYPRRPKPAVSPYPDTLPATLFDSSYDEQQQQLR
LTFQRQHYHHLTDTEHQVLGLPDVMRSDAWGYPAARVPREGFTLEDLLAENSLIAPGTPL
TYLGHQRVAYTGTTGTEEKPTRQALVAYTETAVFDELALQAFNGTLSPEALEKKLIESGY
LSVPRPFNTGAESAVWVARQGYTDYGGSEAFYRPLAQRTTVQIGKNTLHWDTHYCAVVRM
QDAAGLYTDAAYDYRFLTPVQITDANDNQQHITLTALGQVSSGRFWGTEEGTPQGYTPPE
DRPFTPPSSVAEALDLKPDLPVANCMVYAPLSWMPLAHTYQEYIAGFTWQALLDAGVVTE
DKRVCALGFRRWVQRQGIVLNGQALADSREPVHVLTLATDRYDTDPDQQLRKSVTYSDGF
GRLLQSAVYHAPGEAWQRAADGSLITDAKGAPLVAHTATRWAVSGRTEYDGKGQPVRTYP
PTFLNAWQYLSDDSARQDLNADTHRYDPLGREYQVRTAKGYLRQNRLTPWFVVNEDENDT
LS

SEQ ID NO:2

P1-2r

YLPQRGQCDMLLVVIGIGYLNGGQEAVIIGGIRVQTRRILHTDDRTVMGIPMEGVFANLH
RRPLSQRTVKRLRPAVIGISLTGDPDRRFRTGIEWAWNRQITRLD

SEQ ID NO:3

P2-0f

SHLPARYGGRLTTLSRKGFMTVNRGDNLHQKTPEVTVLDNRGLTVRELRYHRHPNTPTTT
DERITRHRFTLSGQLAHSIDPRLFDLQQTDNTVNPNMIYDTALTGEVVRTRSVDAGNDLI
LNDITGRPVLAINATEVTRTWQYENDTLPGRPLSITEQPAGEAGRITERFVWAGNSQAEK
NSNLAGQCVRHYDTAGLNQTDSIALNGIPLSVTRQLLPDGTDADWQGNNEPAWNDRLAPE
NFTTLSTADATGAVLTTTDAAGNLQRVAYDVAGLLTGSWLRLAGGTEQVIVKSLTYSAAG
QKLREEHGNGVVTTYTYEPETQRLVGIKTKRPQGHAQGTKVLQDLRYEYDPVGNVVKVTN
DAEVTRFWRNQKVVPENTYVYDSLYQLVSATGREMANIVQQSTLLPTPSLIDSSTYSNYS
RTYNYDRGDNLTQIRHSAPATGNSYTTDITVSDHSNRAVLDTLTDDPAKVDALFTAGGHQ
IPLQPGQNLVWTPRGELLKVAPVVRDGQISDQESYRYDAASQRIIKTHVQQTANSSQAQS
TLYLPGLERHTTINGTTVKEVLHVITIGEAGRAQVRVLHWENGKPGAISNNQMRYSYDNL
IGSSGLEVDGDGQIISMEEYYPYGGTAVWTARSQTEADYKTVRYSGKERDATGLYYYGYR
YYQPWAGSWLSADPAGTIDGLNLYRMVRNNPATLDDKNGLAPGNRYVFFPFIHEDRIFRL
ASANVYRTEHNKSDIIAVVEDKALDSKLFTNSIEQFFKKPKGKAILKGSPDIKERLLNNI
VHDLSNMQVGDQLYVNAHGHSAKPFFYSDSGYSKIIMEQLQRGANYVAKDLVNKFKLPEN
ATIKISTCHSAEGKGAHITVTSTGTNEKMRYSSIIENKGEFSRSLAGTMENELIKLQPGR

VRGNVYGYLGATTFYGAKNEKVIHLKDGNLTTGVHEGKLSMFTKKNRFSENIFGLKVKRS
LTRTNFTGSGV

SEQ ID NO:4

p-2-9r

PAAEYVRDFTITCSVPPASRSQLPVSRPATSYATRCRLPAASVVVSTAPVASAVLRVVKF
SGASRSFQAGSLFPCQSASVPSGSSWRVTDSGMPLSAILSVWFSPAVS

SEQ ID NO:5

P3-2r

QRALLNDIGHFAPGGTDQLIQAVIDIGVLRHHFLVAPEAGNLRIVRHFHHVPHRVVLIAQ
VLQHLRPLCMSLWAFGFYANKALGLRLVGVGGHHAVAVLFAQFLTRGGIRQGFHDNLLCP
ARKPQPTASQQACYVIRHTLQVTGRIGGGQYRAGGIRRAQGGEVFRCQPVVPGGFIVSLP
VCVRTIRQQLARDGQRYAVKRNTVRLVQSGGVIVTHALSGQVAVLLRLTVPCPDKTLCDT
ACFASRLFCDTERASG

SEQ ID NO:6

P3-6r

SDRRQTGYAYSADHYRISGRSTVCTVRAGLMNYQCWLQHAATQLSESDSPKRDAEILLGY
VTGRSRTYLIAFDETLISSEELHQLDSLLVRRIQGEPVAYIIGEREFWSLPFAVSPATLI
PRPDTECLVEKALELLPDSPARILDLGTGTGAIALALASERNDCYVTGVDINSDAVMLAQ
HNAEKNAGKLAIHNVNFLQSEWFAAVGNQQFDMIVSNPPYIDERDPHLQEGDIRFEPATA
LIAAQNGMADLQAIVGQARHFLSPNGWLLLEHGWKQGTVVRNLFLEKGYQQIATFQDYGG
NERITIGRWNKNETHS

SEQ ID NO:7

P3-7f

ARRAVRRCGYCTGRTESRVPSVTTRCATAMITLSAAAVWRWTVTDKLSVWKNTTRTGALR
CGRRGVRQRLITRLCVTQARSGMQRGCIITATGITSRGRGAG

SEQ ID NO:8

P5-6r

WQNGGSSSTTPRYLAGCYVWYPCSARLSGNAKSLLAPDGEWMKHTLKSKASGNTFTGRLI
PTGRPTVVTIDKSGANTAALTLLNAEGEPQQGIEIRQNKYLNNRIEQDHRHVKRRIRPML
GFKSFRRAQT

SEQ ID NO:9

P5-7r

ALLFLSESRVMSLIRNAFKLLHYPVDIMAQCVRWSLTYALSLRNLEEMMAKRGIFVDHAT
IPRWVLRLVPLLSKAFRKRKKPVGSRWRMDETYIKVKGQWKYLYRSVDTDGQTDCGDYR

SEQ ID NO:10

P6-3f

VHSPSGAVAPGKFFIENFADTFPAPLPLHPFIDACIQQGFQLLPCLIAIAHSGKQAFECV
LLDRLALQGSQCLQALVLPVGDVNGQTAHGFLLIGYTQTHISTYNGLWLFITQGVRYRFV
RQTFVCRSLSFSEDDCTN

SEQ ID NO:11

P6-3r

RTCRERPRLMDYVLTKAAEADLRAIIRHTRKQWGDAQVRRYITALEQGIARLAVGQGSFK
DMSALFPALRMAHCERHYVFCLPRENAPALIVAIFHERMDLLTRLADRLK

SEQ ID NO:12

P6-6r

PQTIICANVGLCITDKEKTMSRLTIDITDRQHQSLKALAALQGKTIKQYALERLFPGMSD
SDQAWQELKALLDTRINEGMEGKGCGKSIGEILDEELAGSDRA

SEQ ID NO:13

P7-1f

NAHFLIVSKTNVVMSNQDPHNKRDSLFSAPIANLGDWSFDERVAEVFPDMVKRSIPGYSN
IISMIGMLASRFVTPGSQIYDLGCSLGAATLSIRRSINADNCRIIAIDNSPAMIERCRRH
IDSFKASTPVEVIEQNILDTDIQNASMVVLNFTLQFLHPDDRQKILKKIYAGLKPGGVLV
LSEKFNFEDQKIGELLFNMHHDFKRANGYSELEVSQKRSMLENVMRTDSVDTHKSRLKEV
GFQHVEVWFQCFNFGSLLAIKGTEQ

SEQ ID NO:14

P7-9f

TMIDFGNFYQLIAKHPLNHWLDSLPAQLSHWQKTSQHGQFSSWVKILENLPEIKPSHLDL
KNGVIAIHEPDLSKGEKARLHNILKILMPWRKGPFSLYDVEIDTEWRSDWKWERVLPHIS
PLEGKTVLDVGCGSGYHMWRMVGEGAQLVVGIDPTQLFLCQFEAIRKLLGNNQRAHLLPL
GIEQLPELQAFDTVFSMGVLYHRRSPLDHLWQLKNQLVSDGELVLESLVIEGDENQCLIP
GERYAQMRNVYFIPSAKMLKVWLEKCGFVDVRIVDHAATTPDEQRRTEWMKTESLVDFLD
PSDHSKTIEGYPAPLRAVLIARKP

SEQ ID NO:15

P8-4r

SLQIDREKVGLDRYPQPIERLRQPCATCDNHCHSRHQVRFFLLKEKYGAALAPISSQSAI
RYQFQRHTMKKGLFAMASIFSGYCGGELFHLLTDPAHESQ

SEQ ID NO:16

P9-8r

SSFRLNDDLLTNSYSEGFLMIKLEICCYSISCALVAQNAGADRIELSASPLEGGLTPSFG
ALQQSLQRLSIPVHPIVRPRGGDFCYNNMDFEAMKNDVARIRDMGFPGIVFGILSENGHI
DRLRMRQLMSLSGNMAVTFHRAFDMCFNPHVALEQLTELGVQRILTSGQQQNAELGLTLL
KELMQASRGPIIMPGAGVRVSNISKFLEAGMTEVHSSAGKIVPSTMKYRKVGVAMSSDDR
DVDEYSHYSVDGELVESMKGVMSLIKR

SEQ ID NO:17

P10-5r

YFGKNRRFVIYVTLMERNFYGLFNGEEMSHFSKISELQDLVADLAGFEQKLKQFEGHLGL
HFEQYSADHISLRCNESKIADRWRKGFLQCGQLISESIINGRPICLFDLNQPIVLLDWKI
DCVELPYPSQKHYVHQGWEHVELVLPVPPEQLICEAKKLLPQFLPDNFRMKESHPKGKNE
RLPNPILAV

SEQ ID NO:18

P10-7f

GNTVNIQVILSEKISNALIEAGAPTDSEAHVRQSAKAQFGDYQANGVMAAAKKVGIPPRQ
LAEKVVSQLDLQGIASKVEIAGPGFINIFLDKAWVAANIETTLKDEKLGITPVEPQTIVI
DYSAPNVAKQMHVGHLRSTIIGDAAARTLEFLGHKVIRANHVGDWGTQFGMLIAYLEKIQ
NENANDMALADLEAFYREAKKHYDEDEEFAIRARNYVVKLQGGDEYCRKMWRKLVDITMS
QNQETYNRLNVTLTEKDVMGESLYNDMLPGIVADLKQRGIAVKSDGATVVYLDEFKNKEG
EPMGVIIQKKDGGYLYTTTDIACAKYRHETLNASRVLYYIDSRQHQHLMQAWAIVRKTGY
IPESMSLEHHMFGMMLGKDGKPFKTRAGGTVRLSDLLDEAIERADTLIREKNPDMPEDEL
KKVVEAVGIGAVKYADLSKSRTTDYVFDWDNMLAFEGNTAPYMQYAYTRVSSIFKRADID
ENSLTLPVMLNEEREQALATRLLQFEETITTVAREGTPHVMCAYLYDLAGLFSGFYEHCP
ILNADSEELRQSRLKLALLTAKTLKQGLDTLGIQTVERM

SEQ ID NO:19

P11-1r

AQVSNMHLLGDIRCGIIDNDGLRFHWGDTELFIFQGSFYICCNPRFIKKNIDKTWACNFN
FAGNSLQIQLADDFFCQLSRRYSHLFSGSHHTIRLIVTKLCFGRLTDVSFTVGWSASFNQ
RIADFF

SEQ ID NO:20

P12-1r

HARVGVLHIRCRVAFKGQHIIPVENIVCSTALGKICIFHRANPYRFHDFFQFVFWHIWVF
LTNEGIRTLNRFIQQIGQSYCAAGTGFEWFTIFAQHHAKHVVFE

SEQ ID NO:21

P12-5r

YHASFQLCRRLLHTFYSLNTQSIKTLLQSFRCQQSQLQAALAQFFAIGIQDRAVLIETRE
QTGQIVQVCTHNMWRTFTGDGSDRFFKLQQAGCQCLLAFFIQHHRQCQAVFIDIRTFKDR

SEQ ID NO:22

P13-1f

FTLREDSMSDWTGVSTFNVILETGLDNCNIYANGLNMIGVIINITPTDDEGNFVDIDDVT
LNDNIKIVDYIDGSDIDGSDGWFYTGNPNEYNTIPNSQSYSLLKSENSQITQIKRYVSCS
NTSRLRTKSFSAKVTTTSGKVISITQNSINSSRVVINAIDATNFTDDELRTTKETRFENQ
SYTSHKSSTNSLYVHTWTIPRSLKLQNWRWEDYNNGWTWAQSCYYKTGADGGSESTRWLA
AGSIFPPGNYDGLWLDNDIALSGMAHKSYNVDTGINQLSFTRIIGKGFSWVYNISGLDRG
HAVIIIDQYGNKYRILFHAGYENSDPYLSSSIVY

SEQ ID NO:23

P14-2f

VYIKFLKLFRRITMSDNNEFFTQANNFTSAVSGGVDPRTGLYNIQITLGHIVGNGNLGPT
LPLTLSYSPLNKTDIGFGIGFNFGLSVYDRKNSLLSLSTGENYKVIETDKTVKLQQKKLD
NLRFEKDLKENCYRIIHKSGDIEVLTGFNNNNAFDLKVPKKLLNPAGHAIYIDWNFEATQP
RLNRIYDDLDGHDIPLLNLEYQGLIKTILTLFPGQKEGYRTELRFLNRQLNSIHNFSLGN
ENPLTWSFGYTPIGKNGILGQWITSMTAPGGLKETVNYSNNNQGHHFPQSANLPVLPYVT
LMKQVPGAGQPAIQAEYSYTSHNYVGGGSNGIWNNKLDNLYGLMTEYNYGSTESRRYKDK
EGHDQIVRIERTYNNYHLLTSECKQQNGYIQTTETAYYAIIGHNFDSQPSQFQLPKTKTE
TWRSADNSYRSEITETTFDESGNPLTKVIKDKKTQKIISPSTHWEYYPPAGEVDNCPPEP
YGFTRFVKKIIQTPYDSEFKDDPEKFIQYRYSLIGSQSHVTLKIEERHYSATQLLNSTLF
QYNTDKSELGRLLKQTECTKGENGKTYSVVHKFTYTKQDDTLQQSHSITTHDNFTIHRSQ
VRSRYTGRLFSDTDTKDIVTQMSYDKLGRLLTRTLNSGTPYANTLTYDYELNNLQDDNRP
PFVITTTDVNGNQLRNEFDGAGRHVSQCLKDSDGDGKFYTIHTQQYDEQGRHHTSTYSDY
LTNGRQQTDPDKVHLSMSKSYDNWGQIANTHWSYGVSEKITVDPITLTATKQLQSNSNNV
QTGKEVTTYTPSQQPIQITLFDEAGHLQSCHTLTRDGWDRVRKETDAIGQCTIYQYDNYN
RVIQITLPDGTIVNRKYAPFSTDTLITDIRVNGISLGQQTFDGLSRLTQSQDGGRVWAYT
YSAGNDQCPSTVITPDGQFIHYQYQPELDDAVLQVASNEITQQFSYNPVTGALLKAVAEG
QSLTPIYYPSGRLKMENINDMKKMSYLWTLRGLENGYTDLTGTIQKISRDTHGRVTQIKD

SSIKTTLNYDDLNRHIGSQVTDLATGHMLTTTVEFDGLNREIGRKLCDSSGHTLDIQQSW
LKTQQLANRIVKLNGVLQRTEQYSYDSRNRLNQYKCDGAECPTDKYGHSIVTQNFTYDIY
GNITACHTTFADGTEDHATFKFANPTDPCQLTEVHHTHPDMPDNIRLKYDKAGRVINITD
NHGNTENFTYDTLGRLQNGQGSVYGYDPLNRLVSQKTDTLDCELYYRETMLVNEVRNGEM
IRLLRTGETIIAQQRASKVLLTGTDSQQSVILTSDKQNLSQEAYSAYGKHKSTANDASIL
GYNGERADPVSGVTHLGNGYRSYDPTLMRFHTPDSLSPFGAGGINPYSYCLGDPINRSDP
SGHLSWQAWTGIGMGIAGLLLTIATGGMAIAAAGGIAAAIASTSTTALAFGALSVTSDIT
SIVSGALEDASPKASSILGWVSMGMGAAGLAESAIKGGTKLATHLGAFAEDGENALLKST
SESSRIKWGVTRSLDREIVRNEEGQVIKDHSRGYTDNFMGKGEQAILVHGDKDGFLYHTE
GNKHNGKGPYTRHTPEQLVDYLKDNNIVDLTQGGDKPVHLLSCYGKSSGAADKMAKYINR
PVIAYSNKPTISQGLARIERKDFFLKSTYHSYDPRKIILGRTEKTVKPKTFRP

SEQ ID NO:24

P17-6r

LCYGHICLSGIPHRHIYIGSTYYGNRKSTVLYAAILHSVSLFYLLIAVFSASSAGYLTYG
LSYHTISVQFLGLSHQIPLLLSTYDQSLNLLLDYQYGDSGHRNLE

SEQ ID NO:25

P17-8r

SAQCIVGKVFRISMVISDIYYSTSLIIFQPDIIRHIWMSVVYLCQLAWVSWVGKFEGSMV
FCPICECGVTGGDIAIDIISKILCDYAMAIFVCRAFRTVTFILVQPITGIVRVLFCTLQY
SIQFHYSIC

SEQ ID NO:26

P18-7r

PSSLRTISLSKLLVTPHFILELSEVDLSKAFSPSSANAPRCVASLVPPLMADSANPAAPI
PIETHPSIEDAFGEASSSAPLTIDVISDVTLSAPNASAVVEVEAIAAAIPPAAAIAIPPV
AMVSSNPAIPMPIPVHACQLK

SEQ ID NO:27

P19-5f

AHCHIALFPCWHNPQYCQQHPDHHSNCHHQFKQEYPPSRQRRENITLTQLPIKHTGIEAG
SQTNRKRQTCMFQRANESKVHQLGQNQGRDRNFYWCFDILT

SEQ ID NO:28

P19-8f

PQSTPSSQNSRQLTPAESSQHQKQKSDHIEIMIPSEAPREYREQLHKATPARNRDVAPNP
SVFDILRDYHWKNFSPVKAAKSSLTPHPVHQKAIPLNDQRNTSMKQSLKPEMRQKLY

SEQ ID NO:29

P20-1r

GKNCINDQGNLPDRYTQNCRPHLTDNPPYGTVTERNPRQYQHADLFQMRKLIGQLQNPSG
NNGPTQRQHWRIAIRSHKQCKNDHTDIEQCRSKSRHRKAVPCIKNCASQRSQRNQKDIRK
RNSK

SEQ ID NO:30

P20-9r

NNTMNLLKSLAAVSSMTMFSRVLGFIRDAIIARIFGAGMATDAFFVAFKLPNLLRRIFAE
GAFSQAFVPILAEYKNQQGDEATRTFIAYISGMLTLILAIVSVIGVIAAPWIIYVTAPGF
TDTPDKFVLTRDLLRITFPYIFLISLASLAGAILNTWNRFSVPAFAPTLLNVSMIIFALF
VAPYCNPPVLALGWAVVAGGVLQLAYQLPHLKKIGMLVLPRISFRDSAVWRVIRQMGPAI
LGVSVGQISLIINTIFASFLVSGSVSWMYYADRLMELPSGVLGVALGTILLPSLAKSFSS
GNHEEYRKLMDWGLRLCFLLALPCAVALGILAEPLTVSLFQYGHFSAFDAEMTQRALIAY
CFGLMGLIVVKVLAPGFYSRQDIKTPVKIAIATLILTQLMNLAFVGPLKHAGLALSIGLA
ACFNASMLYWQLRKRDIFTPLAGWGIFLFKLVVAIAVMVGVLLAVLWVMPAWEQGNMAMR
LLRLMGVVIAGAGSYFAVLALMGFRLKDFAHRGLQ

SEQ ID NO:31

P21-7r

AIILIRDKLSRIFSRQISGEGMFGYRSASPKIRFITDRMVVRLVYERDAYRLAEYYSENK
DFLKPWEPTRDGSFYQPSGWTNRLNYIAELQRQNATFNFVLLDSDEREIMGVANFTNVVR
GAFHSCYLGYSLAEKLQGQGLMYEALQPAIRYMQRYQRMHRIMANYMPHNHRSGNLLKKL
GFEQEGYAKNYLMIDGVWQDHVLTALTDDAWGKVGL

SEQ ID NO:32

P21-8f

WCAMSLVSQARSLGKYFLLFDNLLVVLGFFVVFPLISIRFVEQLGWAALIVGFALGLRQL
VQQGLGIFGGAIADRFGAKPMIVTGMLLRALGFALMAMAHEPWILLLSCVLSGLGGTLFD
PPRAALVIKLTRPHERGRFYSILMMQDSAGAVVGALIGSWLLQYDFNIVCWIGASIFVLA
ALFNAWLLPAYRISTIRTPIKEGMMRVIRDRRFLYYVLTLTGYFVLSVQVMLMFPIIIHE
ITGTPTAVKWMYAIETAISLTLLYPIARWSEKHFRLEQRLMAGLFLMSICMFPIGWVNQL
HTLFGLLCLFYLGLVTADPARETLSASLSDPRARGSYMGFSRLGLALGGAIGYTGGGWLY
DTGRDLNMPQLPWILLGLSGLITIYALHRQFNQKKIDPVMLGRH

SEQ ID NO:33

P23-1f

KGANMKRFFLGAALVLVGLVSGCDQFKDFSINEGLMNDYLLKKVHYQKKISIPGIANANI
TLGDLSSQIGRQDPEKIELSTQAKVQLATLLGTIQADMKLTIKAKPVFDAEKGAIFVKGL
EIVDYQTTPEKAAAPVKALIPYLNTSLSEFFDTHPVYVLNPEKSKAEAAASQFAKRLEIK
PGKLVIGLTDK

SEQ ID NO:34

P24-4r

QVALQHGRRLGTITLFDNLLGLNQVMNEFSIVCRILGTLFNRAPQDPVLQPLITMIAEGK
LKQAWPLEQDEWLDRLQQNSELSVMAADYHALFTGESASVAVCRSDYTDGEESEVRQFLT
ERGMPLSDTPADQFGSLLLAVSWLEDQAAEDEIQAQITLFDEYLLPWCGQFLGKVEAHAT
SGFYRTLAIVTREALQALRDELESE

SEQ ID NO:35

P25-3r

DCMNIIFFHPSFNTDEWIQGIQARLPDAKVRQWVSGDQEPADYALVWQPPYEMLANRQGL
KGIFALGAGVDAIFKQESKNPGTLLADVPLIRLEDTGMGRQMQEYAITSVLHYFRRMDEY
KRYQEQRLWNPIAPHNRKEFVIGVLGAGILGRSVIGKLMEFDFNVRCWSRTSKQLDSVES
FYGKEQLGDFLSGCKVLINLLPDTPDTRGILNLSLFSQLKSGSYVINLARGAQLVEQDLL
VAIDKGYIAGATLDVFAEEPLSNMHPFWTHPRINVTPHIAANTIPEAAMDVICENIRRMV
QGEMPTGLVDRVRGY

SEQ ID NO:36

P26-0f

KTSQGFTSTTCSNGNVLKICGLITPCSSLIQRTYPNNMTIGIFSKESTAKNFGMGFLYYF
DLRVLSPFFKAPINIFTGWQHNTNFRKSRNSTIRLCSSTPNSKQYFTTSRKCHITGAGKY
RFSIENCFIKSG

SEQ ID NO:37

P27-0r

YSAGCSTVLKSSLNLQCDTFNCESFVMLTLNFSTSVNAKPSHIWAHYVDFDLRKKWEVDL
EYFQFEGEVKTGQYGRMILSGMPEIRFYLSNIEVNKEFTDQVNLPQMGILTFRHQIITDE
NNMACRVQVTVSFEPDANIPAVQAESFFKQGTQDLVESVLRLKSVVETVSPKPNLQLVYV
SDIESSTAFYKTIFNAEPIFASSRYVAFPAGGEVLFAIWSGGAKPDRAIPRFSEIGIMLP
SGKDVDRCFEEWRKNPEIKIVQEPHTEVFGRTFLAEDPDGHIIRVCPLD

SEQ ID NO:38

P27-8r

KGNQITMILYKGSKNYLFNQLNYDSCVLLEVDESVNLNGWDELSRAQRLLFLMEILRRYH

FPVQGKVLAQKLNISLRTLYRDIASLQAQGAIIEGEPGIGYVLRPGFVLPPLMFTQNEIE
ALALGANWVAKRADPQLKESANNAISKIAAVIPAELKQMLEASSLLIGPAATAVQPVVEI
QQIRQAINTRHKITLAYLDIKDIPSERTIWPFALGYFENISIVIGWCELREEFRHFRSDR
IMRLKIENQCYPRSRQVLLKEWRAMEKISR

SEQ ID NO:39

P27-9f

RKMTIYDLKPRFQNLLRPIVIYLYKQGITANQVTLTALFLSIFAGSLLSLFPSPHLYWLL
PVFLFIRMALNAIDGMLAREHNQKSHLGAIYNELGDVISDVALYLPFCLLPDVNSLSLLI
ILFLTILTEFIGVLAQTIGASRRYDGPIGKSDRAFIFGAYGLIIAIFPLALGWSISLFAF
MIILLLVTCYQRVVKALREIRLAEQSHSK

SEQ ID NO:40

P28-5f

GVNMTPQLDQRIAEEHYFTTSDNASLFYRYWPQQQANPDRAIIIFHRGHEHSGRIQHVVD
GLDLPDVPMFAWDARGHGKTEGPRGYSPSMGTSIRDVDEFVRFIATQYGIAMENIVVIGQ
SVGAVLVSAWVHDYAPKIRAMILAAPAFDIKLYIPFATQGLQLMQKARGIFFVNSYVKAR
YLTHDETRIASYNSDPLITREIAVNILLDLYQTAERVVKDAAAITLPTLLFISGSDYVVN
KKPQHQFYQQLNTPIKEKHVMDGFYHDTLGEKDRHLVFDKIRVFIERIFALPRYQHDYSQ
EDTWSHSADEFRTLSTSLPCLCPKKLSYQLMRKVMSTHWGRTSEGVCIGLKTGFDSGSTL
DYVYRNQPQGKGILGRILDKHYLNSIGWRGIRQRKIHIEMLIRHAIRSLREQNMPVHMVD
IAAGHGRYILDAINDFSKVDSILLRDYSEINVNQGQAYIEERDLTDKIRFIIGDAFNAES
ISSITPAPTLGIVSGLYELFPDNNLLRNSLRGFADVMTENGYLVYTGQPWHPQIEVIARV
LSSHRDSQPWIMRRRTQGEMDALVEAAGFEKLYQLTDNWGIFTVSIAKRVHR

SEQ ID NO:41

P28-5bf

HHNSINVLLKNIISPHQIMLLCFTVTGHNNRPIQTERSLFFTVVMSTQDVSSMSLTDSIC
LMFLCSRGMPVDTVRQKGRAVTAHPWERRFVMLMNLSDLLPLSTASPWKISWLSARVSER
Y

SEQ ID NO:42

P30-3f

INKYKMEHHMHSSLDSRRRLWLTGVIWLLFLAPFFFLTYGQVNQFTAQRSDVGTVMFGWE
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SRPTTEGLFGWLFNQLELFDLPYNQAPSLHIILLWLLWLRYSAYVSGYWRGLLHIWSVLI
ALSVLTTWQHHFIDVLTGFAVGVILSYLLPVSYRWRWQPNQDRYARKLFGYYLTGSALFA
LIASLLGGSFWILLWPAVSLLMIALGYAGLGSSVFQKQPDGRMSLSARWLLAPYQLGAWL
SYLWFRRKSAPFNHITEGIILGSLPCQPVTAVSVLDITAEWHRRSDARTVNYVCQPQIDL

LPLAPEALQSAVCTLDKLRQQGDVFVHCTLGLSRSAMVVAAWLLKQHPEYDINTVVAILR
KARPHVTFRQTHLDALSQWAKGYL

SEQ ID NO:43

P31-6f

QSCVKPDRMSRSDKHIWMPCLNGQKATYNGEHNMQPENLISKVIIATLKSWRFISTLSAF
SILIATAMLIAVFNTTALNNIALYAVLLFTTLYCQYYCWRTWLDCHYFQILNSSPEKSAE
FDQTLLLIFNKLPQSRTQNDRFNGAIKLLKKATIGLILQWILFFLFLLTLKYSA

SEQ ID NO:44

P32-3f

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LARFGRLIEYIPMSVTLGFTSGIAITIATMQVQNFFGLKLAHIPENYIDKVVALYQALPS
LQLSDTLIGLTTLLVLIFWPKLGVKLPGHLPALIAGTAVMGAMHLLNHDVATIGSSFSYT
LADGTQGQGIPPILPQFVLPWNLPDTHSLDISWNTVSALLPAAFSMAMLGAIESLLCAVI
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SEQ ID NO:45

P33-4r

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MGGMP

SEQ ID NO:46

P33-5f

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FAELREKSADYQTIIMQWDAVPVLDAGGLHAFQGFVRELGKEKHIVVCDIPFQPLKTLAR
AKVMPIEGELSFYATLPKALKEMAVDYTPEVCASSEKIQGQ

SEQ ID NO:47

P34-3f

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NKLWFFNINHSTGFRHRFNQIGLAGKEGWKLNHIHHIRDWLSLCRLMHVSDNFHAEGLFQ
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SEQ ID NO:48

P35-0r

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HEPAQAQPVADVVDVIQLPAFLARQTDLVEAMAKTGAVINVKKPQFVSPGQMGNIVEKFK
EGGNDQVILCDRGSNFGYDNLVVDMLGFGVMQQATQGAPVIFDVTHALQCRDPLGAASGG
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FPELDTSK

SEQ ID NO:49

P35-8r

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VQPVIFPIQLILRIDLLDQPTWFINPLNGDTLNEHTLDVWLKGNIGPTVRLKKQDLQEAD
NVSLVRKITDTIKVSLMEEKKMELALKASEVVLTFDPDDPYEIRDRGLIYAQLDCNHIAV
SDLSYFVEHCPEDPISEMIKMQINTIEQRLIVLH

SEQ ID NO:50

P36-7r

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PRPDTECLVEKALELLPDSPARILDLGTGTGAIALALASERNDCYVTGVDINSDAVMLAQ
HNAEKNAGKLAIHNVNFLQSEWFAAVGNQQFDMIVSNPPYIDERDPHLQEGDIRFEPATA
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NERITIGRWNKNETHS

SEQ ID NO:51

P37-5r

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FRMYSRYAEARRWRVEIISANEGEHGGYKEVIAKVSGDQVYGHLKFESGGHRVQRVPETE
SQGRIHTSACTVAVMPEIPEAELPDISPGDLKIDTFRSSGAGGQHVNTTDSAIRITHLPT
GIVVECQDERSQHKNKAKAMSVLAARIRAAEMRKRQEVEASERRNLLGSGDRSDRNRTYN
FPQGRVTDHRINLTLYRLDEVIEGKLDMLIQPIIIEYQADQLSALSEQD

Claims:

1. The use of a bacterial strain to control a target nematode, characterised in that in nature the bacterial strain is associated symbiotically with an entomopathogenic nematode.

2. The use according to claim 1, wherein the bacterial strain from nature is directly employed to control the nematode target, or is employed to give a recombinant bacterium employed to control the nematode target, or the natural or recombinant strain is employed as a source of a nematode control agent to control the nematode target.

3. The use according to claim 1 or 2, wherein the target nematode is not the same as the nematode with which the bacterial strain is found symbiotically in nature.

4. The use according to claim 1, 2 or 3, for control of helminthiasis in a human or a domesticated animal or the control of plant pathogen nematodes.

5. The use according to any preceding claim wherein the nematode to be controlled comprises one or more of *Haemonchus, Trichostrongylus, Ostertagia, Nematodirus, Cooperia, Ascaris, Bunostomum, Oesophagosromuni, Chaberria, Trichuris, Strongylus, Trichonema, Dictyocaulus, Capillaria, Heterkis, Toxocara, Ascaridia, Oxyuris, Ancylostoma, Uncinaria, Toxascaris, Parascaris, Aphelenochoides, Anguina, Bursaphalenchus, Criconemella, Melodigyne, Ditylenchus, Globodera, Heliocotylenchus, Heterodera, Pratylenchus, Radopholus, Rotelynchus, Tylenchus, Trichodorus, Xiphenema,* and *Caenorhabditis.*

6. A composition for the control of parasitic nematodes which comprises as an effective agent a species of bacterium which is a symbiont of an entomopathogenic nematode, or an engineered bacterium, or a nematode control agent derived from a natural or engineered bacterium.

7. A composition according to claim 6, wherein the bacterial species is of the genera *Xenorhabdus* or *Photorhabdus*,

8. A composition according to claim 7, wherein the bacterial species is of the genus *Xenorhabdus*

9. A composition according to claim 8, wherein the bacterial species is of , the species *Xenorhabdus bovienii*

10. A composition according to claim 8, wherein the bacterial species is:
Xenorhabdus bovienii strain H31 deposited with NCIMB under accession number NCIMB 40985;
Xenorhabdus bovienii strain I73 deposited with NCIMB under accession number NCIMB 40986; and
Xenorhabdus strain C42 deposited with NCIMB under accession number NCIMB 41004.

11. A composition according to any of claim 6, wherein the nematode control agent which is derived from a symbiont of an entomopathogenic nematode or from an engineered bacterium has functional activity against a nematode, and is a peptide.

12. A nucleic acid encoding a peptide of claim 11.

13. A nucleic acid according to claim 12, which nucleic acid comprises a

natural nucleotide sequence or a degeneratively equivalent sequence, or a functional variant thereof.

14. A nucleic acid according to claim 13, which is a homologous variant encoding a peptide which is a nematode control agent, the nucleic acid having 70% or more DNA sequence identity and/or the peptide having 70% or more amino acid sequence identity.

15. A nucleic acid according to claim 13, which is all or part of cosmid cHRIM5, in particvular p 13-1f or p 14-2f, and variants thereof.

16. A nucleic acid according to claim 13, 14 or 15, wherein the variant has a sequence which is a derivative by way of addition, insertion, deletion or substitution of one or more nucleotides.

17. A nucleic acid according to any of claims 12 to 16, which is part of a longer sequence and the nematode control agent is expressed as a fusion protein.

18. A nucleic acid complementary to a nucleic acid according to any of claims 12 to 17.

19. A nucleic acid for use as a probe or primer having a nucleotide sequence of at least 15 nucleotides, which sequence is present in a nucleic acid according to any of claims 12 to 18.

20. A method for identifying or cloning a nucleic acid according to any of claim 12 for a nematode control agent, which method employs a nucleic acid probe according to claim 19.

21. A method according to claim 20, which comprises the steps of:

(a) providing a preparation of nucleic acid from a bacterium,

(b) providing a probe,

(c) contacting nucleic acid in said preparation with said probe under conditions for hybridisation of probe to any said gene or homologue in said preparation, and,

(d) identifying said gene or homologue if present by its hybridisation with said probe.

22. A method according to claim 20, which comprises the use of two primers to amplify a nucleic acid encoding a nematode control agent, at least one of the primers having a conserved nucleotide sequence of at least 15 nucleotides.

23. A method according to claim 20, which comprising the steps of:

(a) providing a preparation of nucleic acid from a bacterium,

(b) providing a pair of nucleic acid molecule primers, at least one of which is a primer,

(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,

(d) performing PCR and determining the presence of absence of an amplified PCR product.

24. A recombinant vector comprising a nucleic acid according to any of claims 12 to 17.

25. A host cell containing a vector according to claim 24 capable of replication.

26. A host cell according to claim 25 which is a plant cell.

27. A method for producing a transgenic plant which comprises the step of regenerating a plant from a plant cell according to claim 26.

28. A plant produced according to claim 27 which is a crop species which can be maize, cotton, soya, rice, *Brassica* species, tomato, potato, sugar beet, barley, soybean, peanut, onion, rye, wheat, corn, banana, raspberry, bean, or a decorative or other plant.

29. A method of producing a peptide nematode control agent comprising causing or allowing expression of a nucleic acid according to claim 12.

30. An antibody or fragment thereof, or a polypeptide comprising the antigen-binding domain of the antibody, capable of specifically binding a peptide of claim 11.

PCT

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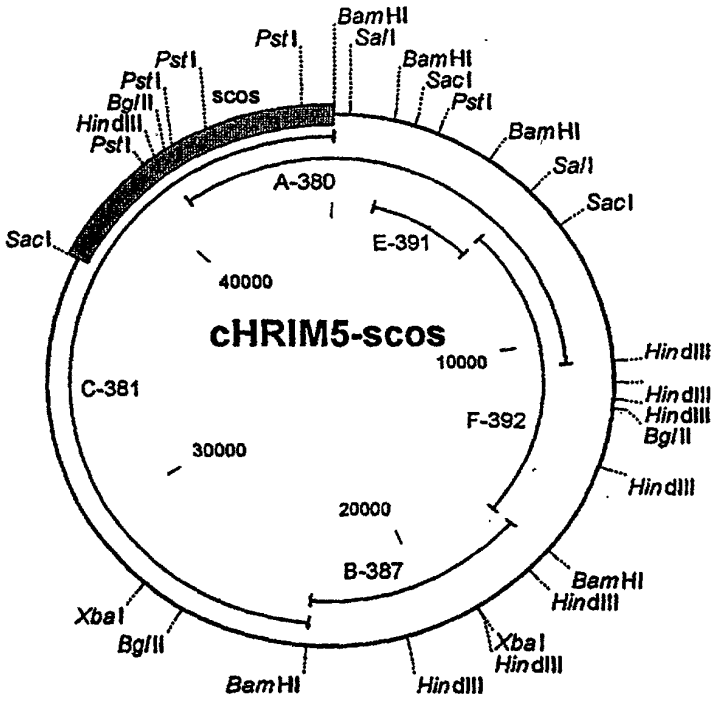
(57) Abstract

Nematodes can be controlled through the use of bacteria associated symbiotically with an entomopathogenic nematode. The bacteria can be employed for nematode control, or engineered to a recombinant form. Control may be achieved using material such as a peptide. The peptide can be obtained from a natural or engineered nucleic acid.



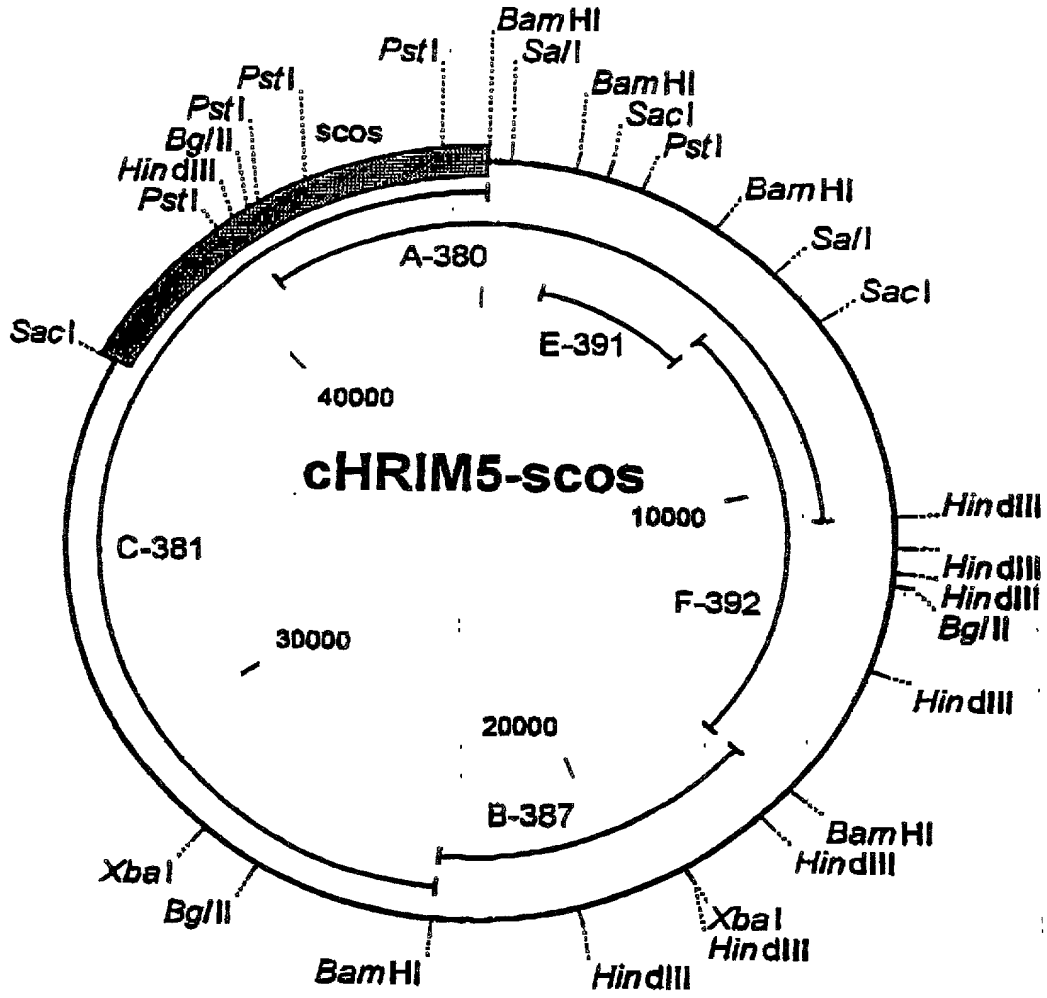


Fig. 1

Sequence Data

Molecule:
Description: chrim5ed2.seq. 37544 bps DNA

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121	ataaagaaca	ggatgttact	ctcgttccct	cgacggaaga	ggcttattgg	ctgcaccggg
181	cgttgcaagg	ccaaccgtta	cacagtgagg	tctatggcga	cgatggcacc	gcgcaggcgg
241	gtatccccta	taccgttatg	gacagtcggc	cccaggttcg	gcttctgacg	ggtttaccgg
301	gtaactcacc	gacagtctgg	ccgagtgtga	ttgaacagag	aacctggcag	tacgaacgga
361	ttgccgatga	tccgcaatgc	catcagcagg	tggtgctgaa	cagtgaccgc	tacggttttc
421	cacgggagac	cgtcgacatt	gcttatccgc	gccgccctaa	gcctgcggtg	tcaccttacc
481	cggatacgct	gccggcgacg	ttattcgaca	gcagctatga	tgagcagcaa	cagcaattgc
541	ggcttacccg	gcaacggcaa	cattaccatc	acctgactga	cactgaacat	caagtgctgg
601	gactgcctga	tgtcatgcga	agcgatgcct	ggggctatcc	ggcagcgcgg	gtaccccgtg
661	aaggtttcac	cctggaggac	ttgctggcag	agaacagtct	gatagccccg	ggcacgccat
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901	atttgtctgt	tccacgccca	ttcaataccg	gtgcggaatc	ggcggtctgg	gtcgcccgtc
961	agggatatac	cgattacggc	gggtctgagg	cgttttaccg	tccgttggct	cagcggacga
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3301	aatctgacgc	agatacgtca	cagtgctccg	gccactggta	acagttacac	cacggacatc
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3661	cacaccacaa	taaatggcac	gacggtgaaa	gaggtgctac	acgttatcac	gataggcgag
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Fig. 2

chrim5ed2.seq

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Fig. 2(i)

chrim5ed2.seq

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8401   tcgatccaac ccaacttttt ctctgtcaat ttgaagcgat cagaaagttg ttggggaaca
8461   atcaacgagc ccaccttctg ccattgggca tcgaacaatt acccgaactg caagcctttg
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8761   gaattgtcga tcatgcggct acaacacctg atgaacagcg ccggacagaa tggatgaaga
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12421  gccagagccg cctgaagttg gctctgctga cagcgaaaac tttgaagcaa ggtcttgata
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Fig. 2(ii)

chrim5ed2.seq

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12781  tgttataaaa aatagtattt taaaaaaaca ttttacatta tataaaatat atcaatcgac
12841  tctttatttc tttatccatt tataaaatat atttttacc aaaataatat ttaaatcata
12901  tattatattt acatcacgtt agatcaaaat aacaattttt tagtcgttaa cccagattca
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16801  cgctgataac agatattcga gtgaatggaa tttccttggg acagcaaacg tttgacgggt
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Fig. 2(iii)

chrim5ed2.seq

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17881 atggttatga tccattaaat cgcttagtga gtcagaaaac agatacccta gattgtgagc
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Fig. 2(iv)

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21361    ccttgcaatt tctcagctaa agaataacca agataacaag agtggaacgc gccgcgtaca
21421    acattagtaa aattcgccac acccataatt tcacgctcat cagagtccaa taatacaaaa
21481    ttaaatgtcg cattctgccg ttgtaactca gcgatatagt tcaaccgatt tgtccatcca
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```

Fig. 2(v)

chrim5ed2.seq

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25681  tgatatcgct acaacaacaa aatacggtga ggtgtccgag aggctgaagg agcacgcctg
25741  gaaagtgtgt atacgtgaaa acgtatcgag ggttcgaacc cctctctcac cgccatattc
25801  taagaaagag cctgaacaca atactaaggc ttttttgtgg ttactcttga tagagcattg
25861  aatctataat ttagtaaccc ttgcggaaaa tccctgacag gacgaccgca gaaacaaaac
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Fig. 2(vi)

chrim5ed2.seq

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Fig. 2(vii)

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Fig. 2(viii)

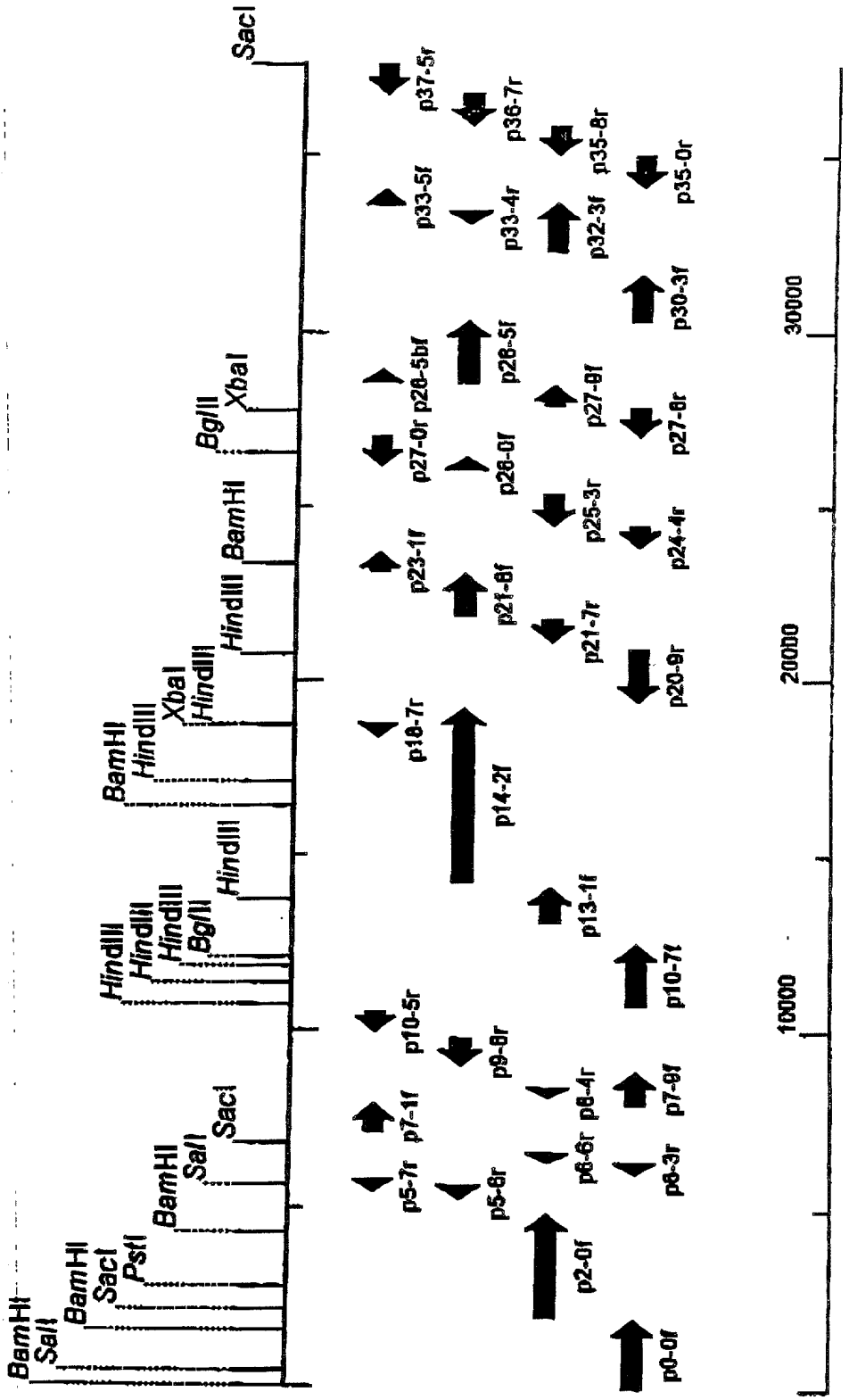


Fig. 3

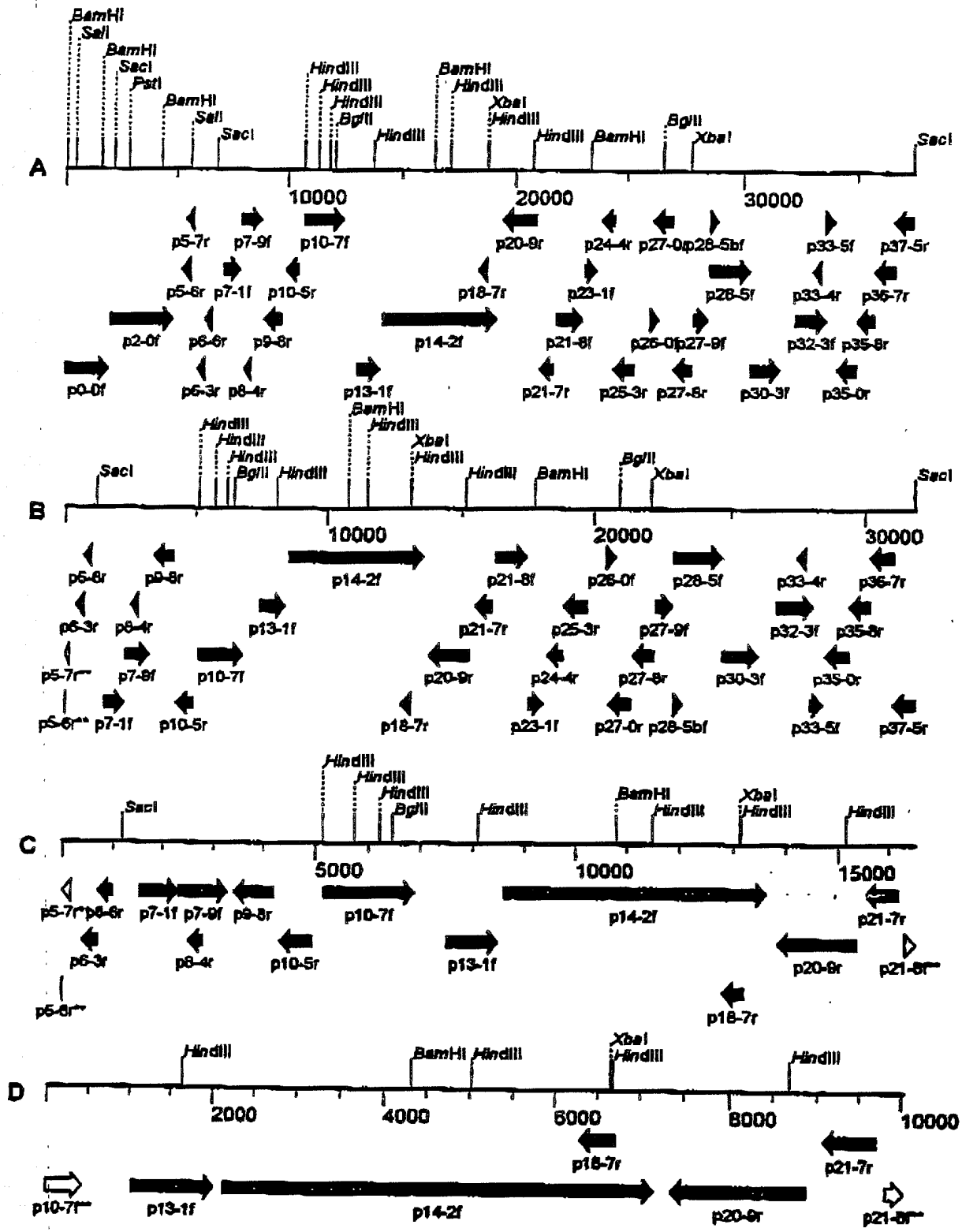
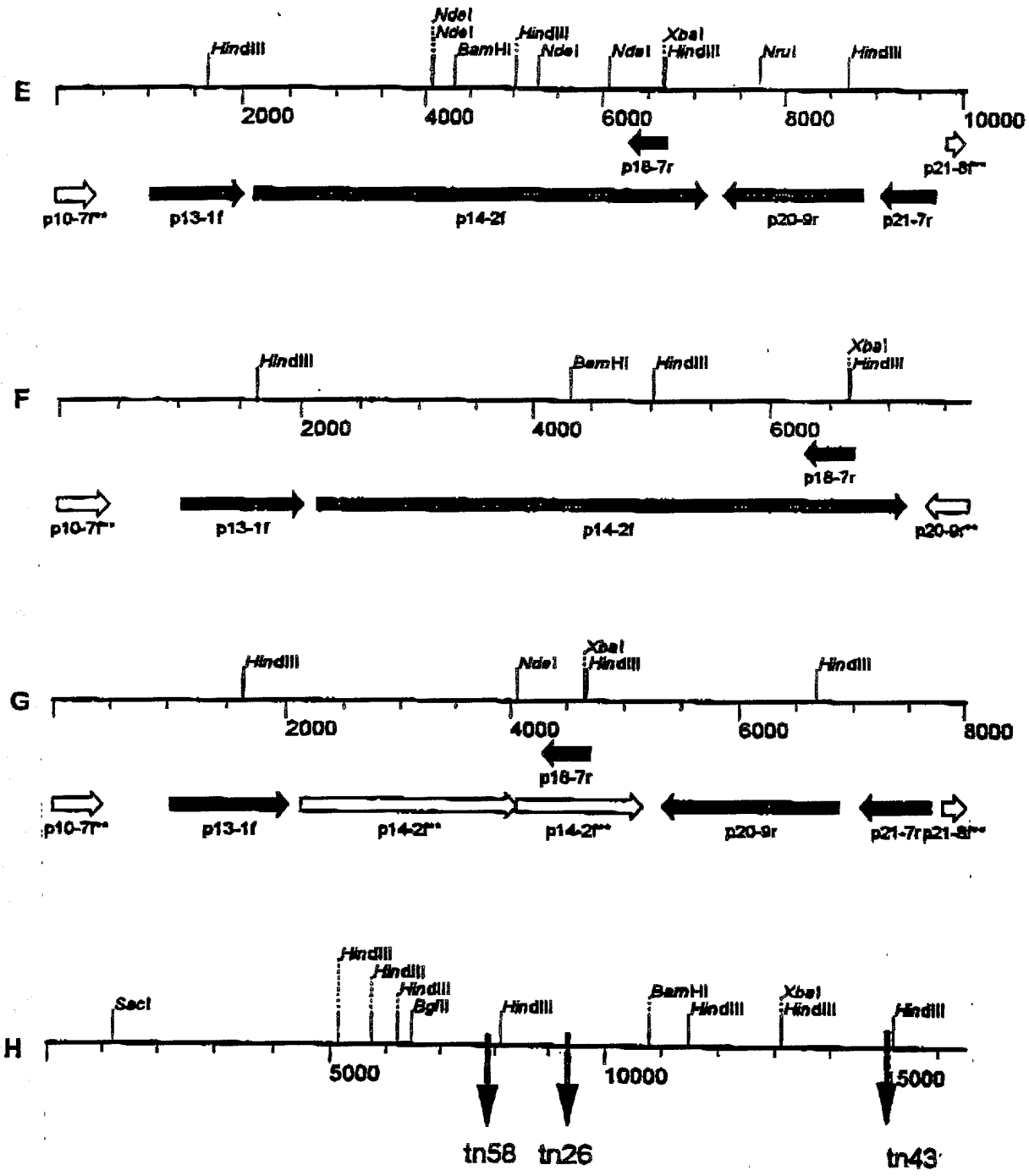


Fig. 4



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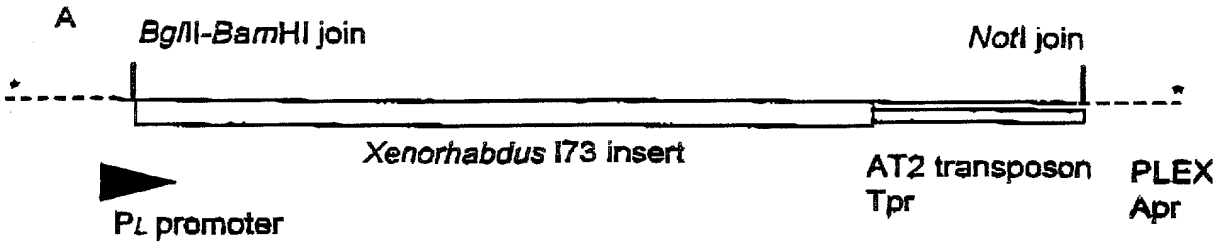

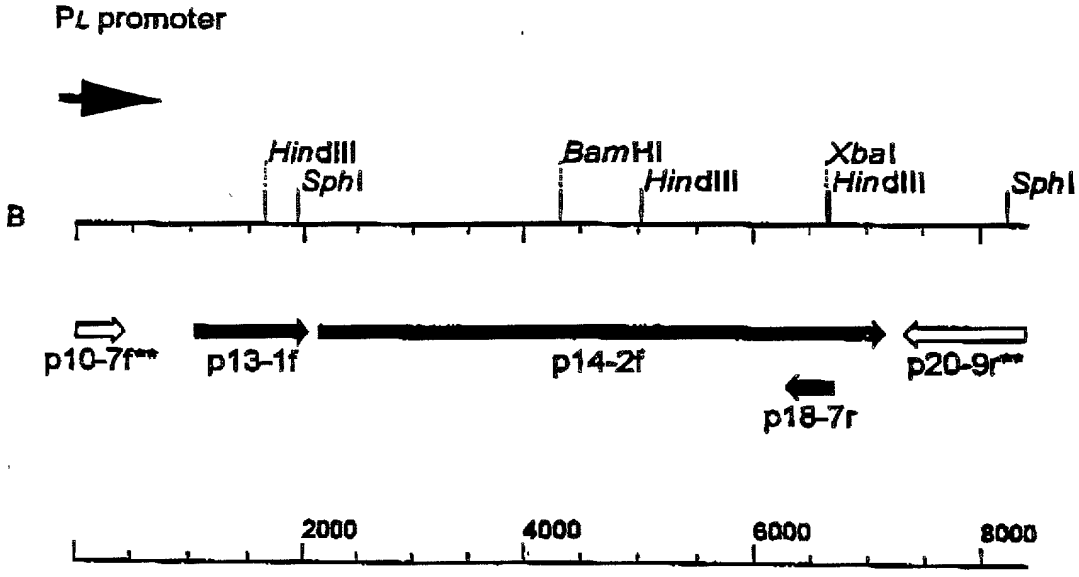

Fig. 5

Attorney's Docket No.: 13384-002001
Client's Ref. No.: F/USP81798

COMBINED DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled BIOLOGICAL CONTROL OF NEMATODES, the specification of which:

[] is attached hereto.
[] was filed on _ as Application Serial No. _ and was amended on _____.
[X] was described and claimed in PCT International Application No. PCT/GB00/00219 filed on 24 January 2000 and as amended under PCT Article 19 on _____.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information I know to be material to patentability in accordance with Title 37, Code of Federal Regulations, §1.56.

I hereby claim the benefit under Title 35, United States Code, §119(e)(1) of any United States provisional application(s) listed below:

U.S. Serial No.	Filing Date	Status

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information I know to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56(a) which became available between the filing date of the prior application and the national or PCT international filing date of this application:

U.S. Serial No.	Filing Date	Status
PCT/GB00/00219	January 24, 2000	Pending

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Country	Application No.	Filing Date	Priority Claimed
Great Britain	9901499.5	22 January 1999	[X] Yes [] No
			[] Yes [] No

I hereby appoint the following attorneys and/or agents to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

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Asn Asp Thr Leu Pro Gly Arg Pro Leu Ser Ile Thr Glu Gln Pro Ala
145 150 155 160
Gly Glu Ala Gly Arg Ile Thr Glu Arg Phe Val Trp Ala Gly Asn Ser
165 170 175
Gln Ala Glu Lys Asn Ser Asn Leu Ala Gly Gln Cys Val Arg His Tyr
180 185 190
Asp Thr Ala Gly Leu Asn Gln Thr Asp Ser Ile Ala Leu Asn Gly Ile
195 200 205
Pro Leu Ser Val Thr Arg Gln Leu Leu Pro Asp Gly Thr Asp Ala Asp

210 215 220
Trp Gln Gly Asn Asn Glu Pro Ala Trp Asn Asp Arg Leu Ala Pro Glu
225 230 235 240
Asn Phe Thr Thr Leu Ser Thr Ala Asp Ala Thr Gly Ala Val Leu Thr
245 250 255
Thr Thr Asp Ala Ala Gly Asn Leu Gln Arg Val Ala Tyr Asp Val Ala
260 265 270
Gly Leu Leu Thr Gly Ser Trp Leu Arg Leu Ala Gly Gly Thr Glu Gln
275 280 285
Val Ile Val Lys Ser Leu Thr Tyr Ser Ala Ala Gly Gln Lys Leu Arg
290 295 300
Glu Glu His Gly Asn Gly Val Val Thr Thr Tyr Thr Tyr Glu Pro Glu
305 310 315 320
Thr Gln Arg Leu Val Gly Ile Lys Thr Lys Arg Pro Gln Gly His Ala
325 330 335
Gln Gly Thr Lys Val Leu Gln Asp Leu Arg Tyr Glu Tyr Asp Pro Val
340 345 350
Gly Asn Val Val Lys Val Thr Asn Asp Ala Glu Val Thr Arg Phe Trp
355 360 365
Arg Asn Gln Lys Val Val Pro Glu Asn Thr Tyr Val Tyr Asp Ser Leu
370 375 380
Tyr Gln Leu Val Ser Ala Thr Gly Arg Glu Met Ala Asn Ile Val Gln
385 390 395 400
Gln Ser Thr Leu Leu Pro Thr Pro Ser Leu Ile Asp Ser Ser Thr Tyr
405 410 415
Ser Asn Tyr Ser Arg Thr Tyr Asn Tyr Asp Arg Gly Asp Asn Leu Thr
420 425 430
Gln Ile Arg His Ser Ala Pro Ala Thr Gly Asn Ser Tyr Thr Thr Asp
435 440 445
Ile Thr Val Ser Asp His Ser Asn Arg Ala Val Leu Asp Thr Leu Thr
450 455 460
Asp Asp Pro Ala Lys Val Asp Ala Leu Phe Thr Ala Gly Gly His Gln
465 470 475 480
Ile Pro Leu Gln Pro Gly Gln Asn Leu Val Trp Thr Pro Arg Gly Glu
485 490 495
Leu Leu Lys Val Ala Pro Val Val Arg Asp Gly Gln Ile Ser Asp Gln
500 505 510
Glu Ser Tyr Arg Tyr Asp Ala Ala Ser Gln Arg Ile Ile Lys Thr His
515 520 525
Val Gln Gln Thr Ala Asn Ser Ser Gln Ala Gln Ser Thr Leu Tyr Leu
530 535 540
Pro Gly Leu Glu Arg His Thr Thr Ile Asn Gly Thr Thr Val Lys Glu
545 550 555 560
Val Leu His Val Ile Thr Ile Gly Glu Ala Gly Arg Ala Gln Val Arg
565 570 575
Val Leu His Trp Glu Asn Gly Lys Pro Gly Ala Ile Ser Asn Asn Gln
580 585 590
Met Arg Tyr Ser Tyr Asp Asn Leu Ile Gly Ser Ser Gly Leu Glu Val
595 600 605
Asp Gly Asp Gly Gln Ile Ile Ser Met Glu Glu Tyr Tyr Pro Tyr Gly
610 615 620
Gly Thr Ala Val Trp Thr Ala Arg Ser Gln Thr Glu Ala Asp Tyr Lys
625 630 635 640
Thr Val Arg Tyr Ser Gly Lys Glu Arg Asp Ala Thr Gly Leu Tyr Tyr
645 650 655
Tyr Gly Tyr Arg Tyr Tyr Gln Pro Trp Ala Gly Ser Trp Leu Ser Ala
660 665 670

Asp Pro Ala Gly Thr Ile Asp Gly Leu Asn Leu Tyr Arg Met Val Arg
        675   680   685
Asn Asn Pro Ala Thr Leu Asp Asp Lys Asn Gly Leu Ala Pro Gly Asn
    690   695   700
Arg Tyr Val Phe Phe Pro Phe Ile His Glu Asp Arg Ile Phe Arg Leu
705   710   715   720
Ala Ser Ala Asn Val Tyr Arg Thr Glu His Asn Lys Ser Asp Ile Ile
                725   730   735
Ala Val Val Glu Asp Lys Ala Leu Asp Ser Lys Leu Phe Thr Asn Ser
            740   745   750
Ile Glu Gln Phe Phe Lys Lys Pro Lys Gly Lys Ala Ile Leu Lys Gly
        755   760   765
Ser Pro Asp Ile Lys Glu Arg Leu Leu Asn Asn Ile Val His Asp Leu
    770   775   780
Ser Asn Met Gln Val Gly Asp Gln Leu Tyr Val Asn Ala His Gly His
785   790   795   800
Ser Ala Lys Pro Phe Phe Tyr Ser Asp Ser Gly Tyr Ser Lys Ile Ile
                805   810   815
Met Glu Gln Leu Gln Arg Gly Ala Asn Tyr Val Ala Lys Asp Leu Val
            820   825   830
Asn Lys Phe Lys Leu Pro Glu Asn Ala Thr Ile Lys Ile Ser Thr Cys
        835   840   845
His Ser Ala Glu Gly Lys Gly Ala His Ile Thr Val Thr Ser Thr Gly
    850   855   860
Thr Asn Glu Lys Met Arg Tyr Ser Ser Ile Ile Glu Asn Lys Gly Glu
865   870   875   880
Phe Ser Arg Ser Leu Ala Gly Thr Met Glu Asn Glu Leu Ile Lys Leu
                885   890   895
Gln Pro Gly Arg Val Arg Gly Asn Val Tyr Gly Tyr Leu Gly Ala Thr
            900   905   910
Thr Phe Tyr Gly Ala Lys Asn Glu Lys Val Ile His Leu Lys Asp Gly
        915   920   925
Asn Leu Thr Thr Gly Val His Glu Gly Lys Leu Ser Met Phe Thr Lys
    930   935   940
Lys Asn Arg Phe Ser Glu Asn Ile Phe Gly Leu Lys Val Lys Arg Ser
945   950   955   960
Leu Thr Arg Thr Asn Phe Thr Gly Ser Gly Val
                965   970

<210> 4
<211> 108
<212> PRT
<213> Xenorhabdus bovienii

<400> 4
Pro Ala Ala Glu Tyr Val Arg Asp Phe Thr Ile Thr Cys Ser Val Pro
1   5   10   15
Pro Ala Ser Arg Ser Gln Leu Pro Val Ser Arg Pro Ala Thr Ser Tyr
            20   25   30
Ala Thr Arg Cys Arg Leu Pro Ala Ala Ser Val Val Val Ser Thr Ala
        35   40   45
Pro Val Ala Ser Ala Val Leu Arg Val Val Lys Phe Ser Gly Ala Ser
    50   55   60
Arg Ser Phe Gln Ala Gly Ser Leu Phe Pro Cys Gln Ser Ala Ser Val
65   70   75   80
Pro Ser Gly Ser Ser Trp Arg Val Thr Asp Ser Gly Met Pro Leu Ser
                85   90   95

Ala Ile Leu Ser Val Trp Phe Ser Pro Ala Val Ser
100 105

<210> 5
<211> 256
<212> PRT
<213> Xenorhabdus bovienii

<400> 5
Gln Arg Ala Leu Leu Asn Asp Ile Gly His Phe Ala Pro Gly Gly Thr
1 5 10 15
Asp Gln Leu Ile Gln Ala Val Ile Asp Ile Gly Val Leu Arg His His
20 25 30
Phe Leu Val Ala Pro Glu Ala Gly Asn Leu Arg Ile Val Arg His Phe
35 40 45
His His Val Pro His Arg Val Val Leu Ile Ala Gln Val Leu Gln His
50 55 60
Leu Arg Pro Leu Cys Met Ser Leu Trp Ala Phe Gly Phe Tyr Ala Asn
65 70 75 80
Lys Ala Leu Gly Leu Arg Leu Val Gly Val Gly Gly His His Ala Val
85 90 95
Ala Val Leu Phe Ala Gln Phe Leu Thr Arg Gly Gly Ile Arg Gln Gly
100 105 110
Phe His Asp Asn Leu Leu Cys Pro Ala Arg Lys Pro Gln Pro Thr Ala
115 120 125
Ser Gln Gln Ala Cys Tyr Val Ile Arg His Thr Leu Gln Val Thr Gly
130 135 140
Arg Ile Gly Gly Gly Gln Tyr Arg Ala Gly Gly Ile Arg Arg Ala Gln
145 150 155 160
Gly Gly Glu Val Phe Arg Cys Gln Pro Val Val Pro Gly Gly Phe Ile
165 170 175
Val Ser Leu Pro Val Cys Val Arg Thr Ile Arg Gln Gln Leu Ala Arg
180 185 190
Asp Gly Gln Arg Tyr Ala Val Lys Arg Asn Thr Val Arg Leu Val Gln
195 200 205
Ser Gly Gly Val Ile Val Thr His Ala Leu Ser Gly Gln Val Ala Val
210 215 220
Leu Leu Arg Leu Thr Val Pro Cys Pro Asp Lys Thr Leu Cys Asp Thr
225 230 235 240
Ala Cys Phe Ala Ser Arg Leu Phe Cys Asp Thr Glu Arg Ala Ser Gly
245 250 255

<210> 6
<211> 316
<212> PRT
<213> Xenorhabdus bovienii

<400> 6
Ser Asp Arg Arg Gln Thr Gly Tyr Ala Tyr Ser Ala Asp His Tyr Arg
1 5 10 15
Ile Ser Gly Arg Ser Thr Val Cys Thr Val Arg Ala Gly Leu Met Asn
20 25 30
Tyr Gln Cys Trp Leu Gln His Ala Ala Thr Gln Leu Ser Glu Ser Asp
35 40 45
Ser Pro Lys Arg Asp Ala Glu Ile Leu Leu Gly Tyr Val Thr Gly Arg
50 55 60
Ser Arg Thr Tyr Leu Ile Ala Phe Asp Glu Thr Leu Ile Ser Ser Glu

65 70 75 80
Glu Leu His Gln Leu Asp Ser Leu Leu Val Arg Arg Ile Gln Gly Glu
85 90 95
Pro Val Ala Tyr Ile Ile Gly Glu Arg Glu Phe Trp Ser Leu Pro Phe
100 105 110
Ala Val Ser Pro Ala Thr Leu Ile Pro Arg Pro Asp Thr Glu Cys Leu
115 120 125
Val Glu Lys Ala Leu Glu Leu Leu Pro Asp Ser Pro Ala Arg Ile Leu
130 135 140
Asp Leu Gly Thr Gly Thr Gly Ala Ile Ala Leu Ala Leu Ala Ser Glu
145 150 155 160
Arg Asn Asp Cys Tyr Val Thr Gly Val Asp Ile Asn Ser Asp Ala Val
165 170 175
Met Leu Ala Gln His Asn Ala Glu Lys Asn Ala Gly Lys Leu Ala Ile
180 185 190
His Asn Val Asn Phe Leu Gln Ser Glu Trp Phe Ala Ala Val Gly Asn
195 200 205
Gln Gln Phe Asp Met Ile Val Ser Asn Pro Pro Tyr Ile Asp Glu Arg
210 215 220
Asp Pro His Leu Gln Glu Gly Asp Ile Arg Phe Glu Pro Ala Thr Ala
225 230 235 240
Leu Ile Ala Ala Gln Asn Gly Met Ala Asp Leu Gln Ala Ile Val Gly
245 250 255
Gln Ala Arg His Phe Leu Ser Pro Asn Gly Trp Leu Leu Leu Glu His
260 265 270
Gly Trp Lys Gln Gly Thr Val Val Arg Asn Leu Phe Leu Glu Lys Gly
275 280 285
Tyr Gln Gln Ile Ala Thr Phe Gln Asp Tyr Gly Gly Asn Glu Arg Ile
290 295 300
Thr Ile Gly Arg Trp Asn Lys Asn Glu Thr His Ser
305 310 315

<210> 7
<211> 102
<212> PRT
<213> Xenorhabdus bovienii

<400> 7
Ala Arg Arg Ala Val Arg Arg Cys Gly Tyr Cys Thr Gly Arg Thr Glu
1 5 10 15
Ser Arg Val Pro Ser Val Thr Thr Arg Cys Ala Thr Ala Met Ile Thr
20 25 30
Leu Ser Ala Ala Ala Val Trp Arg Trp Thr Val Thr Asp Lys Leu Ser
35 40 45
Val Trp Lys Asn Thr Thr Arg Thr Gly Ala Leu Arg Cys Gly Arg Arg
50 55 60
Gly Val Arg Gln Arg Leu Ile Thr Arg Leu Cys Val Thr Gln Ala Arg
65 70 75 80
Ser Gly Met Gln Arg Gly Cys Ile Ile Thr Ala Thr Gly Ile Thr Ser
85 90 95
Arg Gly Arg Gly Ala Gly
100

<210> 8
<211> 130
<212> PRT
<213> Xenorhabdus bovienii

<400> 8
Trp Gln Asn Gly Gly Ser Ser Ser Thr Thr Pro Arg Tyr Leu Ala Gly
1   5   10   15
Cys Tyr Val Trp Tyr Pro Cys Ser Ala Arg Leu Ser Gly Asn Ala Lys
            20   25   30
Ser Leu Leu Ala Pro Asp Gly Glu Trp Met Lys His Thr Leu Lys Ser
        35   40   45
Lys Ala Ser Gly Asn Thr Phe Thr Gly Arg Leu Ile Pro Thr Gly Arg
    50   55   60
Pro Thr Val Val Thr Ile Asp Lys Ser Gly Ala Asn Thr Ala Ala Leu
65   70   75   80
Thr Leu Leu Asn Ala Glu Gly Glu Pro Gln Gln Gly Ile Glu Ile Arg
                85   90   95
Gln Asn Lys Tyr Leu Asn Asn Arg Ile Glu Gln Asp His Arg His Val
            100   105   110
Lys Arg Arg Ile Arg Pro Met Leu Gly Phe Lys Ser Phe Arg Arg Ala
        115   120   125
Gln Thr
    130

<210> 9
<211> 119
<212> PRT
<213> Xenorhabdus bovienii

<400> 9
Ala Leu Leu Phe Leu Ser Glu Ser Arg Val Met Ser Leu Ile Arg Asn
1   5   10   15
Ala Phe Lys Leu Leu His Tyr Pro Val Asp Ile Met Ala Gln Cys Val
            20   25   30
Arg Trp Ser Leu Thr Tyr Ala Leu Ser Leu Arg Asn Leu Glu Glu Met
        35   40   45
Met Ala Lys Arg Gly Ile Phe Val Asp His Ala Thr Ile Pro Arg Trp
    50   55   60
Val Leu Arg Leu Val Pro Leu Leu Ser Lys Ala Phe Arg Lys Arg Lys
65   70   75   80
Lys Pro Val Gly Ser Arg Trp Arg Met Asp Glu Thr Tyr Ile Lys Val
                85   90   95
Lys Gly Gln Trp Lys Tyr Leu Tyr Arg Ser Val Asp Thr Asp Gly Gln
            100   105   110
Thr Asp Cys Gly Asp Tyr Arg
        115

<210> 10
<211> 138
<212> PRT
<213> Xenorhabdus bovienii

<400> 10
Val His Ser Pro Ser Gly Ala Val Ala Pro Gly Lys Phe Phe Ile Glu
1   5   10   15
Asn Phe Ala Asp Thr Phe Pro Ala Pro Leu Pro Leu His Pro Phe Ile
            20   25   30
Asp Ala Cys Ile Gln Gln Gly Phe Gln Leu Leu Pro Cys Leu Ile Ala
        35   40   45
Ile Ala His Ser Gly Lys Gln Ala Phe Glu Cys Val Leu Leu Asp Arg

50 55 60
Leu Ala Leu Gln Gly Ser Gln Cys Leu Gln Ala Leu Val Leu Pro Val
65 70 75 80
Gly Asp Val Asn Gly Gln Thr Ala His Gly Phe Leu Leu Ile Gly Tyr
85 90 95
Thr Gln Thr His Ile Ser Thr Tyr Asn Gly Leu Trp Leu Phe Ile Thr
100 105 110
Gln Gly Val Arg Tyr Arg Phe Val Arg Gln Thr Phe Val Cys Arg Ser
115 120 125
Leu Ser Phe Ser Glu Asp Asp Cys Thr Asn
130 135

<210> 11
<211> 110
<212> PRT
<213> Xenorhabdus bovienii

<400> 11
Arg Thr Cys Arg Glu Arg Pro Arg Leu Met Asp Tyr Val Leu Thr Lys
1 5 10 15
Ala Ala Glu Ala Asp Leu Arg Ala Ile Ile Arg His Thr Arg Lys Gln
20 25 30
Trp Gly Asp Ala Gln Val Arg Arg Tyr Ile Thr Ala Leu Glu Gln Gly
35 40 45
Ile Ala Arg Leu Ala Val Gly Gln Gly Ser Phe Lys Asp Met Ser Ala
50 55 60
Leu Phe Pro Ala Leu Arg Met Ala His Cys Glu Arg His Tyr Val Phe
65 70 75 80
Cys Leu Pro Arg Glu Asn Ala Pro Ala Leu Ile Val Ala Ile Phe His
85 90 95
Glu Arg Met Asp Leu Leu Thr Arg Leu Ala Asp Arg Leu Lys
100 105 110

<210> 12
<211> 103
<212> PRT
<213> Xenorhabdus bovienii

<400> 12
Pro Gln Thr Ile Ile Cys Ala Asn Val Gly Leu Cys Ile Thr Asp Lys
1 5 10 15
Glu Lys Thr Met Ser Arg Leu Thr Ile Asp Ile Thr Asp Arg Gln His
20 25 30
Gln Ser Leu Lys Ala Leu Ala Ala Leu Gln Gly Lys Thr Ile Lys Gln
35 40 45
Tyr Ala Leu Glu Arg Leu Phe Pro Gly Met Ser Asp Ser Asp Gln Ala
50 55 60
Trp Gln Glu Leu Lys Ala Leu Leu Asp Thr Arg Ile Asn Glu Gly Met
65 70 75 80
Glu Gly Lys Gly Cys Gly Lys Ser Ile Gly Glu Ile Leu Asp Glu Glu
85 90 95
Leu Ala Gly Ser Asp Arg Ala
100

<210> 13
<211> 265
<212> PRT

<213> Xenorhabdus bovienii

<400> 13
Asn Ala His Phe Leu Ile Val Ser Lys Thr Asn Val Val Met Ser Asn
1   5   10   15
Gln Asp Pro His Asn Lys Arg Asp Ser Leu Phe Ser Ala Pro Ile Ala
            20   25   30
Asn Leu Gly Asp Trp Ser Phe Asp Glu Arg Val Ala Glu Val Phe Pro
        35   40   45
Asp Met Val Lys Arg Ser Ile Pro Gly Tyr Ser Asn Ile Ile Ser Met
    50   55   60
Ile Gly Met Leu Ala Ser Arg Phe Val Thr Pro Gly Ser Gln Ile Tyr
65   70   75   80
Asp Leu Gly Cys Ser Leu Gly Ala Ala Thr Leu Ser Ile Arg Arg Ser
                85   90   95
Ile Asn Ala Asp Asn Cys Arg Ile Ile Ala Ile Asp Asn Ser Pro Ala
            100   105   110
Met Ile Glu Arg Cys Arg Arg His Ile Asp Ser Phe Lys Ala Ser Thr
        115   120   125
Pro Val Glu Val Ile Glu Gln Asn Ile Leu Asp Thr Asp Ile Gln Asn
    130   135   140
Ala Ser Met Val Val Leu Asn Phe Thr Leu Gln Phe Leu His Pro Asp
145   150   155   160
Asp Arg Gln Lys Ile Leu Lys Lys Ile Tyr Ala Gly Leu Lys Pro Gly
                165   170   175
Gly Val Leu Val Leu Ser Glu Lys Phe Asn Phe Glu Asp Gln Lys Ile
            180   185   190
Gly Glu Leu Leu Phe Asn Met His His Asp Phe Lys Arg Ala Asn Gly
        195   200   205
Tyr Ser Glu Leu Glu Val Ser Gln Lys Arg Ser Met Leu Glu Asn Val
    210   215   220
Met Arg Thr Asp Ser Val Asp Thr His Lys Ser Arg Leu Lys Glu Val
225   230   235   240
Gly Phe Gln His Val Glu Val Trp Phe Gln Cys Phe Asn Phe Gly Ser
                245   250   255
Leu Leu Ala Ile Lys Gly Thr Glu Gln
            260   265

<210> 14
<211> 324
<212> PRT
<213> Xenorhabdus bovienii

<400> 14
Thr Met Ile Asp Phe Gly Asn Phe Tyr Gln Leu Ile Ala Lys His Pro
1   5   10   15
Leu Asn His Trp Leu Asp Ser Leu Pro Ala Gln Leu Ser His Trp Gln
            20   25   30
Lys Thr Ser Gln His Gly Gln Phe Ser Ser Trp Val Lys Ile Leu Glu
        35   40   45
Asn Leu Pro Glu Ile Lys Pro Ser His Leu Asp Leu Lys Asn Gly Val
    50   55   60
Ile Ala Ile His Glu Pro Asp Leu Ser Lys Gly Glu Lys Ala Arg Leu
65   70   75   80
His Asn Ile Leu Lys Ile Leu Met Pro Trp Arg Lys Gly Pro Phe Ser
                85   90   95
Leu Tyr Asp Val Glu Ile Asp Thr Glu Trp Arg Ser Asp Trp Lys Trp

```
            100                 105                 110
Glu Arg Val Leu Pro His Ile Ser Pro Leu Glu Gly Lys Thr Val Leu
        115                 120                 125
Asp Val Gly Cys Gly Ser Gly Tyr His Met Trp Arg Met Val Gly Glu
    130                 135                 140
Gly Ala Gln Leu Val Val Gly Ile Asp Pro Thr Gln Leu Phe Leu Cys
145                 150                 155                 160
Gln Phe Glu Ala Ile Arg Lys Leu Leu Gly Asn Asn Gln Arg Ala His
                165                 170                 175
Leu Leu Pro Leu Gly Ile Glu Gln Leu Pro Glu Leu Gln Ala Phe Asp
            180                 185                 190
Thr Val Phe Ser Met Gly Val Leu Tyr His Arg Arg Ser Pro Leu Asp
        195                 200                 205
His Leu Trp Gln Leu Lys Asn Gln Leu Val Ser Asp Gly Glu Leu Val
    210                 215                 220
Leu Glu Ser Leu Val Ile Glu Gly Asp Glu Asn Gln Cys Leu Ile Pro
225                 230                 235                 240
Gly Glu Arg Tyr Ala Gln Met Arg Asn Val Tyr Phe Ile Pro Ser Ala
                245                 250                 255
Lys Met Leu Lys Val Trp Leu Glu Lys Cys Gly Phe Val Asp Val Arg
            260                 265                 270
Ile Val Asp His Ala Ala Thr Thr Pro Asp Glu Gln Arg Arg Thr Glu
        275                 280                 285
Trp Met Lys Thr Glu Ser Leu Val Asp Phe Leu Asp Pro Ser Asp His
    290                 295                 300
Ser Lys Thr Ile Glu Gly Tyr Pro Ala Pro Leu Arg Ala Val Leu Ile
305                 310                 315                 320
Ala Arg Lys Pro


<210> 15
<211> 100
<212> PRT
<213> Xenorhabdus bovienii

<400> 15
Ser Leu Gln Ile Asp Arg Glu Lys Val Gly Leu Asp Arg Tyr Pro Gln
 1               5                  10                  15
Pro Ile Glu Arg Leu Arg Gln Pro Cys Ala Thr Cys Asp Asn His Cys
            20                  25                  30
His Ser Arg His Gln Val Arg Phe Phe Leu Leu Lys Glu Lys Tyr Gly
        35                  40                  45
Ala Ala Leu Ala Pro Ile Ser Ser Gln Ser Ala Ile Arg Tyr Gln Phe
    50                  55                  60
Gln Arg His Thr Met Lys Lys Gly Leu Phe Ala Met Ala Ser Ile Phe
65                  70                  75                  80
Ser Gly Tyr Cys Gly Gly Glu Leu Phe His Leu Leu Thr Asp Pro Ala
                85                  90                  95
His Glu Ser Gln
            100

<210> 16
<211> 267
<212> PRT
<213> Xenorhabdus bovienii

<400> 16
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```
Ser Ser Phe Arg Leu Asn Asp Asp Leu Leu Thr Asn Ser Tyr Ser Glu
 1               5                  10                  15
Gly Phe Leu Met Ile Lys Leu Glu Ile Cys Cys Tyr Ser Ile Ser Cys
            20                  25                  30
Ala Leu Val Ala Gln Asn Ala Gly Ala Asp Arg Ile Glu Leu Ser Ala
        35                  40                  45
Ser Pro Leu Glu Gly Gly Leu Thr Pro Ser Phe Gly Ala Leu Gln Gln
    50                  55                  60
Ser Leu Gln Arg Leu Ser Ile Pro Val His Pro Ile Val Arg Pro Arg
65                  70                  75                  80
Gly Gly Asp Phe Cys Tyr Asn Asn Met Asp Phe Glu Ala Met Lys Asn
                85                  90                  95
Asp Val Ala Arg Ile Arg Asp Met Gly Phe Pro Gly Ile Val Phe Gly
            100                 105                 110
Ile Leu Ser Glu Asn Gly His Ile Asp Arg Leu Arg Met Arg Gln Leu
        115                 120                 125
Met Ser Leu Ser Gly Asn Met Ala Val Thr Phe His Arg Ala Phe Asp
    130                 135                 140
Met Cys Phe Asn Pro His Val Ala Leu Glu Gln Leu Thr Glu Leu Gly
145                 150                 155                 160
Val Gln Arg Ile Leu Thr Ser Gly Gln Gln Gln Asn Ala Glu Leu Gly
                165                 170                 175
Leu Thr Leu Leu Lys Glu Leu Met Gln Ala Ser Arg Gly Pro Ile Ile
            180                 185                 190
Met Pro Gly Ala Gly Val Arg Val Ser Asn Ile Ser Lys Phe Leu Glu
        195                 200                 205
Ala Gly Met Thr Glu Val His Ser Ser Ala Gly Lys Ile Val Pro Ser
    210                 215                 220
Thr Met Lys Tyr Arg Lys Val Gly Val Ala Met Ser Ser Asp Asp Arg
225                 230                 235                 240
Asp Val Asp Glu Tyr Ser His Tyr Ser Val Asp Gly Glu Leu Val Glu
                245                 250                 255
Ser Met Lys Gly Val Met Ser Leu Ile Lys Arg
            260                 265
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<210> 17
<211> 189
<212> PRT
<213> Xenorhabdus bovienii

<400> 17

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Tyr Phe Gly Lys Asn Arg Arg Phe Val Ile Tyr Val Thr Leu Met Glu
 1               5                  10                  15
Arg Asn Phe Tyr Gly Leu Phe Asn Gly Glu Glu Met Ser His Phe Ser
            20                  25                  30
Lys Ile Ser Glu Leu Gln Asp Leu Val Ala Asp Leu Ala Gly Phe Glu
        35                  40                  45
Gln Lys Leu Lys Gln Phe Glu Gly His Leu Gly Leu His Phe Glu Gln
    50                  55                  60
Tyr Ser Ala Asp His Ile Ser Leu Arg Cys Asn Glu Ser Lys Ile Ala
65                  70                  75                  80
Asp Arg Trp Arg Lys Gly Phe Leu Gln Cys Gly Gln Leu Ile Ser Glu
                85                  90                  95
Ser Ile Ile Asn Gly Arg Pro Ile Cys Leu Phe Asp Leu Asn Gln Pro
            100                 105                 110
Ile Val Leu Leu Asp Trp Lys Ile Asp Cys Val Glu Leu Pro Tyr Pro
        115                 120                 125
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Ser Gln Lys His Tyr Val His Gln Gly Trp Glu His Val Glu Leu Val
    130                 135                 140
Leu Pro Val Pro Pro Glu Gln Leu Ile Cys Glu Ala Lys Lys Leu Leu
145                 150                 155                 160
Pro Gln Pro Leu Pro Asp Asn Phe Arg Met Lys Glu Ser His Pro Lys
                165                 170                 175
Gly Lys Asn Glu Arg Leu Pro Asn Pro Ile Leu Ala Val
            180                 185

<210> 18
<211> 579
<212> PRT
<213> Xenorhabdus bovienii

<400> 18
Gly Asn Thr Val Asn Ile Gln Val Ile Leu Ser Glu Lys Ile Ser Asn
 1               5                  10                  15
Ala Leu Ile Glu Ala Gly Ala Pro Thr Asp Ser Glu Ala His Val Arg
            20                  25                  30
Gln Ser Ala Lys Ala Gln Phe Gly Asp Tyr Gln Ala Asn Gly Val Met
        35                  40                  45
Ala Ala Ala Lys Lys Val Gly Ile Pro Pro Arg Gln Leu Ala Glu Lys
    50                  55                  60
Val Val Ser Gln Leu Asp Leu Gln Gly Ile Ala Ser Lys Val Glu Ile
65                  70                  75                  80
Ala Gly Pro Gly Phe Ile Asn Ile Phe Leu Asp Lys Ala Trp Val Ala
                85                  90                  95
Ala Asn Ile Glu Thr Thr Leu Lys Asp Glu Lys Leu Gly Ile Thr Pro
            100                 105                 110
Val Glu Pro Gln Thr Ile Val Ile Asp Tyr Ser Ala Pro Asn Val Ala
        115                 120                 125
Lys Gln Met His Val Gly His Leu Arg Ser Thr Ile Ile Gly Asp Ala
    130                 135                 140
Ala Ala Arg Thr Leu Glu Phe Leu Gly His Lys Val Ile Arg Ala Asn
145                 150                 155                 160
His Val Gly Asp Trp Gly Thr Gln Phe Gly Met Leu Ile Ala Tyr Leu
                165                 170                 175
Glu Lys Ile Gln Asn Glu Asn Ala Asn Asp Met Ala Leu Ala Asp Leu
            180                 185                 190
Glu Ala Phe Tyr Arg Glu Ala Lys Lys His Tyr Asp Glu Asp Glu Glu
        195                 200                 205
Phe Ala Ile Arg Ala Arg Asn Tyr Val Val Lys Leu Gln Gly Gly Asp
    210                 215                 220
Glu Tyr Cys Arg Lys Met Trp Arg Lys Leu Val Asp Ile Thr Met Ser
225                 230                 235                 240
Gln Asn Gln Glu Thr Tyr Asn Arg Leu Asn Val Thr Leu Thr Glu Lys
                245                 250                 255
Asp Val Met Gly Glu Ser Leu Tyr Asn Asp Met Leu Pro Gly Ile Val
            260                 265                 270
Ala Asp Leu Lys Gln Arg Gly Ile Ala Val Lys Ser Asp Gly Ala Thr
        275                 280                 285
Val Val Tyr Leu Asp Glu Phe Lys Asn Lys Glu Gly Glu Pro Met Gly
    290                 295                 300
Val Ile Ile Gln Lys Lys Asp Gly Gly Tyr Leu Tyr Thr Thr Thr Asp
305                 310                 315                 320
Ile Ala Cys Ala Lys Tyr Arg His Glu Thr Leu Asn Ala Ser Arg Val
                325                 330                 335
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Leu Tyr Tyr Ile Asp Ser Arg Gln His Gln His Leu Met Gln Ala Trp
340 345 350
Ala Ile Val Arg Lys Thr Gly Tyr Ile Pro Glu Ser Met Ser Leu Glu
355 360 365
His His Met Phe Gly Met Met Leu Gly Lys Asp Gly Lys Pro Phe Lys
370 375 380
Thr Arg Ala Gly Gly Thr Val Arg Leu Ser Asp Leu Leu Asp Glu Ala
385 390 395 400
Ile Glu Arg Ala Asp Thr Leu Ile Arg Glu Lys Asn Pro Asp Met Pro
405 410 415
Glu Asp Glu Leu Lys Lys Val Val Glu Ala Val Gly Ile Gly Ala Val
420 425 430
Lys Tyr Ala Asp Leu Ser Lys Ser Arg Thr Thr Asp Tyr Val Phe Asp
435 440 445
Trp Asp Asn Met Leu Ala Phe Glu Gly Asn Thr Ala Pro Tyr Met Gln
450 455 460
Tyr Ala Tyr Thr Arg Val Ser Ser Ile Phe Lys Arg Ala Asp Ile Asp
465 470 475 480
Glu Asn Ser Leu Thr Leu Pro Val Met Leu Asn Glu Glu Arg Glu Gln
485 490 495
Ala Leu Ala Thr Arg Leu Leu Gln Phe Glu Glu Thr Ile Thr Thr Val
500 505 510
Ala Arg Glu Gly Thr Pro His Val Met Cys Ala Tyr Leu Tyr Asp Leu
515 520 525
Ala Gly Leu Phe Ser Gly Phe Tyr Glu His Cys Pro Ile Leu Asn Ala
530 535 540
Asp Ser Glu Glu Leu Arg Gln Ser Arg Leu Lys Leu Ala Leu Leu Thr
545 550 555 560
Ala Lys Thr Leu Lys Gln Gly Leu Asp Thr Leu Gly Ile Gln Thr Val
565 570 575
Glu Arg Met

<210> 19
<211> 126
<212> PRT
<213> Xenorhabdus bovienii

<400> 19
Ala Gln Val Ser Asn Met His Leu Leu Gly Asp Ile Arg Cys Gly Ile
1 5 10 15
Ile Asp Asn Asp Gly Leu Arg Phe His Trp Gly Asp Thr Glu Leu Phe
20 25 30
Ile Phe Gln Gly Ser Phe Tyr Ile Cys Cys Asn Pro Arg Phe Ile Lys
35 40 45
Lys Asn Ile Asp Lys Thr Trp Ala Cys Asn Phe Asn Phe Ala Gly Asn
50 55 60
Ser Leu Gln Ile Gln Leu Ala Asp Asp Phe Phe Cys Gln Leu Ser Arg
65 70 75 80
Arg Tyr Ser His Leu Phe Ser Gly Ser His His Thr Ile Arg Leu Ile
85 90 95
Val Thr Lys Leu Cys Phe Gly Arg Leu Thr Asp Val Ser Phe Thr Val
100 105 110
Gly Trp Ser Ala Ser Phe Asn Gln Arg Ile Ala Asp Phe Phe
115 120 125

<210> 20

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<211> 104
<212> PRT
<213> Xenorhabdus bovienii

<400> 20
His Ala Arg Val Gly Val Leu His Ile Arg Cys Arg Val Ala Phe Lys
 1               5                  10                  15
Gly Gln His Ile Ile Pro Val Glu Asn Ile Val Cys Ser Thr Ala Leu
            20                  25                  30
Gly Lys Ile Cys Ile Phe His Arg Ala Asn Pro Tyr Arg Phe His Asp
        35                  40                  45
Phe Phe Gln Phe Val Phe Trp His Ile Trp Val Phe Leu Thr Asn Glu
    50                  55                  60
Gly Ile Arg Thr Leu Asn Arg Phe Ile Gln Gln Ile Gly Gln Ser Tyr
65                  70                  75                  80
Cys Ala Ala Gly Thr Gly Phe Glu Trp Phe Thr Ile Phe Ala Gln His
                85                  90                  95
His Ala Lys His Val Val Phe Glu
            100

<210> 21
<211> 120
<212> PRT
<213> Xenorhabdus bovienii

<400> 21
Tyr His Ala Ser Phe Gln Leu Cys Arg Arg Leu Leu His Thr Phe Tyr
 1               5                  10                  15
Ser Leu Asn Thr Gln Ser Ile Lys Thr Leu Leu Gln Ser Phe Arg Cys
            20                  25                  30
Gln Gln Ser Gln Leu Gln Ala Ala Leu Ala Gln Phe Phe Ala Ile Gly
        35                  40                  45
Ile Gln Asp Arg Ala Val Leu Ile Glu Thr Arg Glu Gln Thr Gly Gln
    50                  55                  60
Ile Val Gln Val Cys Thr His Asn Met Trp Arg Thr Phe Thr Gly Asp
65                  70                  75                  80
Gly Ser Asp Arg Phe Phe Lys Leu Gln Gln Ala Gly Cys Gln Cys Leu
                85                  90                  95
Leu Ala Phe Phe Ile Gln His His Arg Gln Cys Gln Ala Val Phe Ile
            100                 105                 110
Asp Ile Arg Thr Phe Lys Asp Arg
        115                 120

<210> 22
<211> 334
<212> PRT
<213> Xenorhabdus bovienii

<400> 22
Phe Thr Leu Arg Glu Asp Ser Met Ser Asp Trp Thr Gly Val Ser Thr
 1               5                  10                  15
Phe Asn Val Ile Leu Glu Thr Gly Leu Asp Asn Cys Asn Ile Tyr Ala
            20                  25                  30
Asn Gly Leu Asn Met Ile Gly Val Ile Ile Asn Ile Thr Pro Thr Asp
        35                  40                  45
Asp Glu Gly Asn Phe Val Asp Ile Asp Asp Val Thr Leu Asn Asp Asn
    50                  55                  60
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```
Ile Lys Ile Val Asp Tyr Ile Asp Gly Ser Asp Ile Asp Gly Ser Asp
65                  70                  75                  80
Gly Trp Phe Tyr Thr Gly Asn Pro Asn Glu Tyr Asn Thr Ile Pro Asn
                85                  90                  95
Ser Gln Ser Tyr Ser Leu Leu Lys Ser Glu Asn Ser Gln Ile Thr Gln
            100                 105                 110
Ile Lys Arg Tyr Val Ser Cys Ser Asn Thr Ser Arg Leu Arg Thr Lys
        115                 120                 125
Ser Phe Ser Ala Lys Val Thr Thr Thr Ser Gly Lys Val Ile Ser Ile
    130                 135                 140
Thr Gln Asn Ser Ile Asn Ser Ser Arg Val Val Ile Asn Ala Ile Asp
145                 150                 155                 160
Ala Thr Asn Phe Thr Asp Asp Glu Leu Arg Thr Thr Lys Glu Thr Arg
                165                 170                 175
Phe Glu Asn Gln Ser Tyr Thr Ser His Lys Ser Ser Thr Asn Ser Leu
            180                 185                 190
Tyr Val His Thr Trp Thr Ile Pro Arg Ser Leu Lys Leu Gln Asn Trp
        195                 200                 205
Arg Trp Glu Asp Tyr Asn Asn Gly Trp Thr Trp Ala Gln Ser Cys Tyr
    210                 215                 220
Tyr Lys Thr Gly Ala Asp Gly Gly Ser Glu Ser Thr Arg Trp Leu Ala
225                 230                 235                 240
Ala Gly Ser Ile Phe Pro Pro Gly Asn Tyr Asp Gly Leu Trp Leu Asp
                245                 250                 255
Asn Asp Ile Ala Leu Ser Gly Met Ala His Lys Ser Tyr Asn Val Asp
            260                 265                 270
Thr Gly Ile Asn Gln Leu Ser Phe Thr Arg Ile Ile Gly Lys Gly Phe
        275                 280                 285
Ser Trp Val Tyr Asn Ile Ser Gly Leu Asp Arg Gly His Ala Val Ile
    290                 295                 300
Ile Ile Asp Gln Tyr Gly Asn Lys Tyr Arg Ile Leu Phe His Ala Gly
305                 310                 315                 320
Tyr Glu Asn Ser Asp Pro Tyr Leu Ser Ser Ser Ile Val Tyr
                325                 330
```

<210> 23
<211> 1673
<212> PRT
<213> Xenorhabdus bovienii

<400> 23

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Val Tyr Ile Lys Phe Leu Lys Leu Phe Arg Arg Ile Thr Met Ser Asp
 1               5                  10                  15
Asn Asn Glu Phe Phe Thr Gln Ala Asn Asn Phe Thr Ser Ala Val Ser
            20                  25                  30
Gly Gly Val Asp Pro Arg Thr Gly Leu Tyr Asn Ile Gln Ile Thr Leu
        35                  40                  45
Gly His Ile Val Gly Asn Gly Asn Leu Gly Pro Thr Leu Pro Leu Thr
    50                  55                  60
Leu Ser Tyr Ser Pro Leu Asn Lys Thr Asp Ile Gly Phe Gly Ile Gly
65                  70                  75                  80
Phe Asn Phe Gly Leu Ser Val Tyr Asp Arg Lys Asn Ser Leu Leu Ser
                85                  90                  95
Leu Ser Thr Gly Glu Asn Tyr Lys Val Ile Glu Thr Asp Lys Thr Val
            100                 105                 110
Lys Leu Gln Gln Lys Lys Leu Asp Asn Leu Arg Phe Glu Lys Asp Leu
        115                 120                 125
```

Lys Glu Asn Cys Tyr Arg Ile Ile His Lys Ser Gly Asp Ile Glu Val
130 135 140

Leu Thr Gly Phe Asn Asn Asn Ala Phe Asp Leu Lys Val Pro Lys Lys
145 150 155 160

Leu Leu Asn Pro Ala Gly His Ala Ile Tyr Ile Asp Trp Asn Phe Glu
165 170 175

Ala Thr Gln Pro Arg Leu Asn Arg Ile Tyr Asp Asp Leu Asp Gly His
180 185 190

Asp Ile Pro Leu Leu Asn Leu Glu Tyr Gln Gly Leu Ile Lys Thr Ile
195 200 205

Leu Thr Leu Phe Pro Gly Gln Lys Glu Gly Tyr Arg Thr Glu Leu Arg
210 215 220

Phe Leu Asn Arg Gln Leu Asn Ser Ile His Asn Phe Ser Leu Gly Asn
225 230 235 240

Glu Asn Pro Leu Thr Trp Ser Phe Gly Tyr Thr Pro Ile Gly Lys Asn
245 250 255

Gly Ile Leu Gly Gln Trp Ile Thr Ser Met Thr Ala Pro Gly Gly Leu
260 265 270

Lys Glu Thr Val Asn Tyr Ser Asn Asn Asn Gln Gly His His Phe Pro
275 280 285

Gln Ser Ala Asn Leu Pro Val Leu Pro Tyr Val Thr Leu Met Lys Gln
290 295 300

Val Pro Gly Ala Gly Gln Pro Ala Ile Gln Ala Glu Tyr Ser Tyr Thr
305 310 315 320

Ser His Asn Tyr Val Gly Gly Gly Ser Asn Gly Ile Trp Asn Asn Lys
325 330 335

Leu Asp Asn Leu Tyr Gly Leu Met Thr Glu Tyr Asn Tyr Gly Ser Thr
340 345 350

Glu Ser Arg Arg Tyr Lys Asp Lys Glu Gly His Asp Gln Ile Val Arg
355 360 365

Ile Glu Arg Thr Tyr Asn Asn Tyr His Leu Leu Thr Ser Glu Cys Lys
370 375 380

Gln Gln Asn Gly Tyr Ile Gln Thr Thr Glu Thr Ala Tyr Tyr Ala Ile
385 390 395 400

Ile Gly His Asn Phe Asp Ser Gln Pro Ser Gln Phe Gln Leu Pro Lys
405 410 415

Thr Lys Thr Glu Thr Trp Arg Ser Ala Asp Asn Ser Tyr Arg Ser Glu
420 425 430

Ile Thr Glu Thr Thr Phe Asp Glu Ser Gly Asn Pro Leu Thr Lys Val
435 440 445

Ile Lys Asp Lys Lys Thr Gln Lys Ile Ile Ser Pro Ser Thr His Trp
450 455 460

Glu Tyr Tyr Pro Pro Ala Gly Glu Val Asp Asn Cys Pro Pro Glu Pro
465 470 475 480

Tyr Gly Phe Thr Arg Phe Val Lys Lys Ile Ile Gln Thr Pro Tyr Asp
485 490 495

Ser Glu Phe Lys Asp Asp Pro Glu Lys Phe Ile Gln Tyr Arg Tyr Ser
500 505 510

Leu Ile Gly Ser Gln Ser His Val Thr Leu Lys Ile Glu Glu Arg His
515 520 525

Tyr Ser Ala Thr Gln Leu Leu Asn Ser Thr Leu Phe Gln Tyr Asn Thr
530 535 540

Asp Lys Ser Glu Leu Gly Arg Leu Leu Lys Gln Thr Glu Cys Thr Lys
545 550 555 560

Gly Glu Asn Gly Lys Thr Tyr Ser Val Val His Lys Phe Thr Tyr Thr
565 570 575

Lys Gln Asp Asp Thr Leu Gln Gln Ser His Ser Ile Thr Thr His Asp

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            580                 585                 590
Asn Phe Thr Ile His Arg Ser Gln Val Arg Ser Arg Tyr Thr Gly Arg
        595                 600                 605
Leu Phe Ser Asp Thr Asp Thr Lys Asp Ile Val Thr Gln Met Ser Tyr
    610                 615                 620
Asp Lys Leu Gly Arg Leu Leu Thr Arg Thr Leu Asn Ser Gly Thr Pro
625                 630                 635                 640
Tyr Ala Asn Thr Leu Thr Tyr Asp Tyr Glu Leu Asn Asn Leu Gln Asp
                645                 650                 655
Asp Asn Arg Pro Pro Phe Val Ile Thr Thr Thr Asp Val Asn Gly Asn
            660                 665                 670
Gln Leu Arg Asn Glu Phe Asp Gly Ala Gly Arg His Val Ser Gln Cys
        675                 680                 685
Leu Lys Asp Ser Asp Gly Asp Gly Lys Phe Tyr Thr Ile His Thr Gln
    690                 695                 700
Gln Tyr Asp Glu Gln Gly Arg His His Thr Ser Thr Tyr Ser Asp Tyr
705                 710                 715                 720
Leu Thr Asn Gly Arg Gln Gln Thr Asp Pro Asp Lys Val His Leu Ser
                725                 730                 735
Met Ser Lys Ser Tyr Asp Asn Trp Gly Gln Ile Ala Asn Thr His Trp
            740                 745                 750
Ser Tyr Gly Val Ser Glu Lys Ile Thr Val Asp Pro Ile Thr Leu Thr
        755                 760                 765
Ala Thr Lys Gln Leu Gln Ser Asn Ser Asn Asn Val Gln Thr Gly Lys
    770                 775                 780
Glu Val Thr Thr Tyr Thr Pro Ser Gln Gln Pro Ile Gln Ile Thr Leu
785                 790                 795                 800
Phe Asp Glu Ala Gly His Leu Gln Ser Cys His Thr Leu Thr Arg Asp
                805                 810                 815
Gly Trp Asp Arg Val Arg Lys Glu Thr Asp Ala Ile Gly Gln Cys Thr
            820                 825                 830
Ile Tyr Gln Tyr Asp Asn Tyr Asn Arg Val Ile Gln Ile Thr Leu Pro
        835                 840                 845
Asp Gly Thr Ile Val Asn Arg Lys Tyr Ala Pro Phe Ser Thr Asp Thr
    850                 855                 860
Leu Ile Thr Asp Ile Arg Val Asn Gly Ile Ser Leu Gly Gln Gln Thr
865                 870                 875                 880
Phe Asp Gly Leu Ser Arg Leu Thr Gln Ser Gln Asp Gly Gly Arg Val
                885                 890                 895
Trp Ala Tyr Thr Tyr Ser Ala Gly Asn Asp Gln Cys Pro Ser Thr Val
            900                 905                 910
Ile Thr Pro Asp Gly Gln Phe Ile His Tyr Gln Tyr Gln Pro Glu Leu
        915                 920                 925
Asp Asp Ala Val Leu Gln Val Ala Ser Asn Glu Ile Thr Gln Gln Phe
    930                 935                 940
Ser Tyr Asn Pro Val Thr Gly Ala Leu Leu Lys Ala Val Ala Glu Gly
945                 950                 955                 960
Gln Ser Leu Thr Pro Ile Tyr Tyr Pro Ser Gly Arg Leu Lys Met Glu
                965                 970                 975
Asn Ile Asn Asp Met Lys Lys Met Ser Tyr Leu Trp Thr Leu Arg Gly
            980                 985                 990
Leu Glu Asn Gly Tyr Thr Asp Leu Thr Gly Thr Ile Gln Lys Ile Ser
        995                 1000                1005
Arg Asp Thr His Gly Arg Val Thr Gln Ile Lys Asp Ser Ser Ile Lys
    1010                1015                1020
Thr Thr Leu Asn Tyr Asp Asp Leu Asn Arg His Ile Gly Ser Gln Val
1025                1030                1035                1040
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Thr Asp Leu Ala Thr Gly His Met Leu Thr Thr Thr Val Glu Phe Asp
                1045                1050                1055
Gly Leu Asn Arg Glu Ile Gly Arg Lys Leu Cys Asp Ser Ser Gly His
            1060                1065                1070
Thr Leu Asp Ile Gln Gln Ser Trp Leu Lys Thr Gln Gln Leu Ala Asn
        1075                1080                1085
Arg Ile Val Lys Leu Asn Gly Val Leu Gln Arg Thr Glu Gln Tyr Ser
    1090                1095                1100
Tyr Asp Ser Arg Asn Arg Leu Asn Gln Tyr Lys Cys Asp Gly Ala Glu
1105                1110                1115                1120
Cys Pro Thr Asp Lys Tyr Gly His Ser Ile Val Thr Gln Asn Phe Thr
                1125                1130                1135
Tyr Asp Ile Tyr Gly Asn Ile Thr Ala Cys His Thr Thr Phe Ala Asp
            1140                1145                1150
Gly Thr Glu Asp His Ala Thr Phe Lys Phe Ala Asn Pro Thr Asp Pro
        1155                1160                1165
Cys Gln Leu Thr Glu Val His His Thr His Pro Asp Met Pro Asp Asn
    1170                1175                1180
Ile Arg Leu Lys Tyr Asp Lys Ala Gly Arg Val Ile Asn Ile Thr Asp
1185                1190                1195                1200
Asn His Gly Asn Thr Glu Asn Phe Thr Tyr Asp Thr Leu Gly Arg Leu
                1205                1210                1215
Gln Asn Gly Gln Gly Ser Val Tyr Gly Tyr Asp Pro Leu Asn Arg Leu
            1220                1225                1230
Val Ser Gln Lys Thr Asp Thr Leu Asp Cys Glu Leu Tyr Tyr Arg Glu
        1235                1240                1245
Thr Met Leu Val Asn Glu Val Arg Asn Gly Glu Met Ile Arg Leu Leu
    1250                1255                1260
Arg Thr Gly Glu Thr Ile Ile Ala Gln Gln Arg Ala Ser Lys Val Leu
1265                1270                1275                1280
Leu Thr Gly Thr Asp Ser Gln Gln Ser Val Ile Leu Thr Ser Asp Lys
                1285                1290                1295
Gln Asn Leu Ser Gln Glu Ala Tyr Ser Ala Tyr Gly Lys His Lys Ser
            1300                1305                1310
Thr Ala Asn Asp Ala Ser Ile Leu Gly Tyr Asn Gly Glu Arg Ala Asp
        1315                1320                1325
Pro Val Ser Gly Val Thr His Leu Gly Asn Gly Tyr Arg Ser Tyr Asp
    1330                1335                1340
Pro Thr Leu Met Arg Phe His Thr Pro Asp Ser Leu Ser Pro Phe Gly
1345                1350                1355                1360
Ala Gly Gly Ile Asn Pro Tyr Ser Tyr Cys Leu Gly Asp Pro Ile Asn
                1365                1370                1375
Arg Ser Asp Pro Ser Gly His Leu Ser Trp Gln Ala Trp Thr Gly Ile
            1380                1385                1390
Gly Met Gly Ile Ala Gly Leu Leu Leu Thr Ile Ala Thr Gly Gly Met
        1395                1400                1405
Ala Ile Ala Ala Ala Gly Gly Ile Ala Ala Ala Ile Ala Ser Thr Ser
    1410                1415                1420
Thr Thr Ala Leu Ala Phe Gly Ala Leu Ser Val Thr Ser Asp Ile Thr
1425                1430                1435                1440
Ser Ile Val Ser Gly Ala Leu Glu Asp Ala Ser Pro Lys Ala Ser Ser
                1445                1450                1455
Ile Leu Gly Trp Val Ser Met Gly Met Gly Ala Ala Gly Leu Ala Glu
            1460                1465                1470
Ser Ala Ile Lys Gly Gly Thr Lys Leu Ala Thr His Leu Gly Ala Phe
        1475                1480                1485
Ala Glu Asp Gly Glu Asn Ala Leu Leu Lys Ser Thr Ser Glu Ser Ser
```

1490 1495 1500
Arg Ile Lys Trp Gly Val Thr Arg Ser Leu Asp Arg Glu Ile Val Arg
1505 1510 1515 1520
Asn Glu Glu Gly Gln Val Ile Lys Asp His Ser Arg Gly Tyr Thr Asp
1525 1530 1535
Asn Phe Met Gly Lys Gly Glu Gln Ala Ile Leu Val His Gly Asp Lys
1540 1545 1550
Asp Gly Phe Leu Tyr His Thr Glu Gly Asn Lys His Asn Gly Lys Gly
1555 1560 1565
Pro Tyr Thr Arg His Thr Pro Glu Gln Leu Val Asp Tyr Leu Lys Asp
1570 1575 1580
Asn Asn Ile Val Asp Leu Thr Gln Gly Gly Asp Lys Pro Val His Leu
1585 1590 1595 1600
Leu Ser Cys Tyr Gly Lys Ser Ser Gly Ala Ala Asp Lys Met Ala Lys
1605 1610 1615
Tyr Ile Asn Arg Pro Val Ile Ala Tyr Ser Asn Lys Pro Thr Ile Ser
1620 1625 1630
Gln Gly Leu Ala Arg Ile Glu Arg Lys Asp Phe Phe Leu Lys Ser Thr
1635 1640 1645
Tyr His Ser Tyr Asp Pro Arg Lys Ile Ile Leu Gly Arg Thr Glu Lys
1650 1655 1660
Thr Val Lys Pro Lys Thr Phe Arg Pro
1665 1670

<210> 24
<211> 105
<212> PRT
<213> Xenorhabdus bovienii

<400> 24
Leu Cys Tyr Gly His Ile Cys Leu Ser Gly Ile Pro His Arg His Ile
1 5 10 15
Tyr Ile Gly Ser Thr Tyr Tyr Gly Asn Arg Lys Ser Thr Val Leu Tyr
20 25 30
Ala Ala Ile Leu His Ser Val Ser Leu Phe Tyr Leu Leu Ile Ala Val
35 40 45
Phe Ser Ala Ser Ser Ala Gly Tyr Leu Thr Tyr Gly Leu Ser Tyr His
50 55 60
Thr Ile Ser Val Gln Phe Leu Gly Leu Ser His Gln Ile Pro Leu Leu
65 70 75 80
Leu Ser Thr Tyr Asp Gln Ser Leu Asn Leu Leu Leu Asp Tyr Gln Tyr
85 90 95
Gly Asp Ser Gly His Arg Asn Leu Glu
100 105

<210> 25
<211> 129
<212> PRT
<213> Xenorhabdus bovienii

<400> 25
Ser Ala Gln Cys Ile Val Gly Lys Val Phe Arg Ile Ser Met Val Ile
1 5 10 15
Ser Asp Ile Tyr Tyr Ser Thr Ser Leu Ile Ile Phe Gln Pro Asp Ile
20 25 30
Ile Arg His Ile Trp Met Ser Val Val Tyr Leu Cys Gln Leu Ala Trp
35 40 45

```
Val Ser Trp Val Gly Lys Phe Glu Gly Ser Met Val Phe Cys Pro Ile
    50                  55                  60
Cys Glu Cys Gly Val Thr Gly Gly Asp Ile Ala Ile Asp Ile Ile Ser
65                  70                  75                  80
Lys Ile Leu Cys Asp Tyr Ala Met Ala Ile Phe Val Cys Arg Ala Phe
                85                  90                  95
Arg Thr Val Thr Phe Ile Leu Val Gln Pro Ile Thr Gly Ile Val Arg
            100                 105                 110
Val Leu Phe Cys Thr Leu Gln Tyr Ser Ile Gln Phe His Tyr Ser Ile
        115                 120                 125
Cys
```

<210> 26
<211> 141
<212> PRT
<213> Xenorhabdus bovienii

<400> 26

```
Pro Ser Ser Leu Arg Thr Ile Ser Leu Ser Lys Leu Leu Val Thr Pro
 1               5                  10                  15
His Phe Ile Leu Glu Leu Ser Glu Val Asp Leu Ser Lys Ala Phe Ser
            20                  25                  30
Pro Ser Ser Ala Asn Ala Pro Arg Cys Val Ala Ser Leu Val Pro Pro
        35                  40                  45
Leu Met Ala Asp Ser Ala Asn Pro Ala Ala Pro Ile Pro Ile Glu Thr
    50                  55                  60
His Pro Ser Ile Glu Asp Ala Phe Gly Glu Ala Ser Ser Ser Ala Pro
65                  70                  75                  80
Leu Thr Ile Asp Val Ile Ser Asp Val Thr Leu Ser Ala Pro Asn Ala
                85                  90                  95
Ser Ala Val Val Glu Val Glu Ala Ile Ala Ala Ala Ile Pro Pro Ala
            100                 105                 110
Ala Ala Ile Ala Ile Pro Pro Val Ala Met Val Ser Ser Asn Pro Ala
        115                 120                 125
Ile Pro Met Pro Ile Pro Val His Ala Cys Gln Leu Lys
    130                 135                 140
```

<210> 27
<211> 101
<212> PRT
<213> Xenorhabdus bovienii

<400> 27

```
Ala His Cys His Ile Ala Leu Phe Pro Cys Trp His Asn Pro Gln Tyr
 1               5                  10                  15
Cys Gln Gln His Pro Asp His His Ser Asn Cys His His Gln Phe Lys
            20                  25                  30
Gln Glu Tyr Pro Pro Ser Arg Gln Arg Arg Glu Asn Ile Thr Leu Thr
        35                  40                  45
Gln Leu Pro Ile Lys His Thr Gly Ile Glu Ala Gly Ser Gln Thr Asn
    50                  55                  60
Arg Lys Arg Gln Thr Cys Met Phe Gln Arg Ala Asn Glu Ser Lys Val
65                  70                  75                  80
His Gln Leu Gly Gln Asn Gln Gly Arg Asp Arg Asn Phe Tyr Trp Cys
                85                  90                  95
Phe Asp Ile Leu Thr
```

100

<210> 28
<211> 117
<212> PRT
<213> Xenorhabdus bovienii

<400> 28
Pro Gln Ser Thr Pro Ser Ser Gln Asn Ser Arg Gln Leu Thr Pro Ala
1 5 10 15
Glu Ser Ser Gln His Gln Lys Gln Lys Ser Asp His Ile Glu Ile Met
20 25 30
Ile Pro Ser Glu Ala Pro Arg Glu Tyr Arg Glu Gln Leu His Lys Ala
35 40 45
Thr Pro Ala Arg Asn Arg Asp Val Ala Pro Asn Pro Ser Val Phe Asp
50 55 60
Ile Leu Arg Asp Tyr His Trp Lys Asn Phe Ser Pro Val Lys Ala Ala
65 70 75 80
Lys Ser Ser Leu Thr Pro His Pro Val His Gln Lys Ala Ile Pro Leu
85 90 95
Asn Asp Gln Arg Asn Thr Ser Met Lys Gln Ser Leu Lys Pro Glu Met
100 105 110
Arg Gln Lys Leu Tyr
115

<210> 29
<211> 124
<212> PRT
<213> Xenorhabdus bovienii

<400> 29
Gly Lys Asn Cys Ile Asn Asp Gln Gly Asn Leu Pro Asp Arg Tyr Thr
1 5 10 15
Gln Asn Cys Arg Pro His Leu Thr Asp Asn Pro Pro Tyr Gly Thr Val
20 25 30
Thr Glu Arg Asn Pro Arg Gln Tyr Gln His Ala Asp Leu Phe Gln Met
35 40 45
Arg Lys Leu Ile Gly Gln Leu Gln Asn Pro Ser Gly Asn Asn Gly Pro
50 55 60
Thr Gln Arg Gln His Trp Arg Ile Ala Ile Arg Ser His Lys Gln Cys
65 70 75 80
Lys Asn Asp His Thr Asp Ile Glu Gln Cys Arg Ser Lys Ser Arg His
85 90 95
Arg Lys Ala Val Pro Cys Ile Lys Asn Cys Ala Ser Gln Arg Ser Gln
100 105 110
Arg Asn Gln Lys Asp Ile Arg Lys Arg Asn Ser Lys
115 120

<210> 30
<211> 515
<212> PRT
<213> Xenorhabdus bovienii

<400> 30
Asn Asn Thr Met Asn Leu Leu Lys Ser Leu Ala Ala Val Ser Ser Met
1 5 10 15
Thr Met Phe Ser Arg Val Leu Gly Phe Ile Arg Asp Ala Ile Ile Ala

```
            20                  25                  30
Arg Ile Phe Gly Ala Gly Met Ala Thr Asp Ala Phe Phe Val Ala Phe
        35                  40                  45
Lys Leu Pro Asn Leu Leu Arg Arg Ile Phe Ala Glu Gly Ala Phe Ser
    50                  55                  60
Gln Ala Phe Val Pro Ile Leu Ala Glu Tyr Lys Asn Gln Gln Gly Asp
65                  70                  75                  80
Glu Ala Thr Arg Thr Phe Ile Ala Tyr Ile Ser Gly Met Leu Thr Leu
                85                  90                  95
Ile Leu Ala Ile Val Ser Val Ile Gly Val Ile Ala Ala Pro Trp Ile
            100                 105                 110
Ile Tyr Val Thr Ala Pro Gly Phe Thr Asp Thr Pro Asp Lys Phe Val
        115                 120                 125
Leu Thr Arg Asp Leu Leu Arg Ile Thr Phe Pro Tyr Ile Phe Leu Ile
    130                 135                 140
Ser Leu Ala Ser Leu Ala Gly Ala Ile Leu Asn Thr Trp Asn Arg Phe
145                 150                 155                 160
Ser Val Pro Ala Phe Ala Pro Thr Leu Leu Asn Val Ser Met Ile Ile
                165                 170                 175
Phe Ala Leu Phe Val Ala Pro Tyr Cys Asn Pro Pro Val Leu Ala Leu
            180                 185                 190
Gly Trp Ala Val Val Ala Gly Gly Val Leu Gln Leu Ala Tyr Gln Leu
        195                 200                 205
Pro His Leu Lys Lys Ile Gly Met Leu Val Leu Pro Arg Ile Ser Phe
    210                 215                 220
Arg Asp Ser Ala Val Trp Arg Val Ile Arg Gln Met Gly Pro Ala Ile
225                 230                 235                 240
Leu Gly Val Ser Val Gly Gln Ile Ser Leu Ile Ile Asn Thr Ile Phe
                245                 250                 255
Ala Ser Phe Leu Val Ser Gly Ser Val Ser Trp Met Tyr Tyr Ala Asp
            260                 265                 270
Arg Leu Met Glu Leu Pro Ser Gly Val Leu Gly Val Ala Leu Gly Thr
        275                 280                 285
Ile Leu Leu Pro Ser Leu Ala Lys Ser Phe Ser Ser Gly Asn His Glu
    290                 295                 300
Glu Tyr Arg Lys Leu Met Asp Trp Gly Leu Arg Leu Cys Phe Leu Leu
305                 310                 315                 320
Ala Leu Pro Cys Ala Val Ala Leu Gly Ile Leu Ala Glu Pro Leu Thr
                325                 330                 335
Val Ser Leu Phe Gln Tyr Gly His Phe Ser Ala Phe Asp Ala Glu Met
            340                 345                 350
Thr Gln Arg Ala Leu Ile Ala Tyr Cys Phe Gly Leu Met Gly Leu Ile
        355                 360                 365
Val Val Lys Val Leu Ala Pro Gly Phe Tyr Ser Arg Gln Asp Ile Lys
    370                 375                 380
Thr Pro Val Lys Ile Ala Ile Ala Thr Leu Ile Leu Thr Gln Leu Met
385                 390                 395                 400
Asn Leu Ala Phe Val Gly Pro Leu Lys His Ala Gly Leu Ala Leu Ser
                405                 410                 415
Ile Gly Leu Ala Ala Cys Phe Asn Ala Ser Met Leu Tyr Trp Gln Leu
            420                 425                 430
Arg Lys Arg Asp Ile Phe Thr Pro Leu Ala Gly Trp Gly Ile Phe Leu
        435                 440                 445
Phe Lys Leu Val Val Ala Ile Ala Val Met Val Gly Val Leu Leu Ala
    450                 455                 460
Val Leu Trp Val Met Pro Ala Trp Glu Gln Gly Asn Met Ala Met Arg
465                 470                 475                 480
```

Leu Leu Arg Leu Met Gly Val Val Ile Ala Gly Ala Gly Ser Tyr Phe
485 490 495
Ala Val Leu Ala Leu Met Gly Phe Arg Leu Lys Asp Phe Ala His Arg
500 505 510
Gly Leu Gln
515

<210> 31
<211> 216
<212> PRT
<213> Xenorhabdus bovienii

<400> 31
Ala Ile Ile Leu Ile Arg Asp Lys Leu Ser Arg Ile Phe Ser Arg Gln
1 5 10 15
Ile Ser Gly Glu Gly Met Phe Gly Tyr Arg Ser Ala Ser Pro Lys Ile
20 25 30
Arg Phe Ile Thr Asp Arg Met Val Val Arg Leu Val Tyr Glu Arg Asp
35 40 45
Ala Tyr Arg Leu Ala Glu Tyr Tyr Ser Glu Asn Lys Asp Phe Leu Lys
50 55 60
Pro Trp Glu Pro Thr Arg Asp Gly Ser Phe Tyr Gln Pro Ser Gly Trp
65 70 75 80
Thr Asn Arg Leu Asn Tyr Ile Ala Glu Leu Gln Arg Gln Asn Ala Thr
85 90 95
Phe Asn Phe Val Leu Leu Asp Ser Asp Glu Arg Glu Ile Met Gly Val
100 105 110
Ala Asn Phe Thr Asn Val Val Arg Gly Ala Phe His Ser Cys Tyr Leu
115 120 125
Gly Tyr Ser Leu Ala Glu Lys Leu Gln Gly Gln Gly Leu Met Tyr Glu
130 135 140
Ala Leu Gln Pro Ala Ile Arg Tyr Met Gln Arg Tyr Gln Arg Met His
145 150 155 160
Arg Ile Met Ala Asn Tyr Met Pro His Asn His Arg Ser Gly Asn Leu
165 170 175
Leu Lys Lys Leu Gly Phe Glu Gln Glu Gly Tyr Ala Lys Asn Tyr Leu
180 185 190
Met Ile Asp Gly Val Trp Gln Asp His Val Leu Thr Ala Leu Thr Asp
195 200 205
Asp Ala Trp Gly Lys Val Gly Leu
210 215

<210> 32
<211> 404
<212> PRT
<213> Xenorhabdus bovienii

<400> 32
Trp Cys Ala Met Ser Leu Val Ser Gln Ala Arg Ser Leu Gly Lys Tyr
1 5 10 15
Phe Leu Leu Phe Asp Asn Leu Leu Val Val Leu Gly Phe Phe Val Val
20 25 30
Phe Pro Leu Ile Ser Ile Arg Phe Val Glu Gln Leu Gly Trp Ala Ala
35 40 45
Leu Ile Val Gly Phe Ala Leu Gly Leu Arg Gln Leu Val Gln Gln Gly
50 55 60
Leu Gly Ile Phe Gly Gly Ala Ile Ala Asp Arg Phe Gly Ala Lys Pro

```
65                  70                  75                  80
Met Ile Val Thr Gly Met Leu Leu Arg Ala Leu Gly Phe Ala Leu Met
                85                  90                  95
Ala Met Ala His Glu Pro Trp Ile Leu Leu Leu Ser Cys Val Leu Ser
            100                 105                 110
Gly Leu Gly Gly Thr Leu Phe Asp Pro Pro Arg Ala Ala Leu Val Ile
        115                 120                 125
Lys Leu Thr Arg Pro His Glu Arg Gly Arg Phe Tyr Ser Ile Leu Met
    130                 135                 140
Met Gln Asp Ser Ala Gly Ala Val Val Gly Ala Leu Ile Gly Ser Trp
145                 150                 155                 160
Leu Leu Gln Tyr Asp Phe Asn Ile Val Cys Trp Ile Gly Ala Ser Ile
                165                 170                 175
Phe Val Leu Ala Ala Leu Phe Asn Ala Trp Leu Leu Pro Ala Tyr Arg
            180                 185                 190
Ile Ser Thr Ile Arg Thr Pro Ile Lys Glu Gly Met Met Arg Val Ile
        195                 200                 205
Arg Asp Arg Arg Phe Leu Tyr Tyr Val Leu Thr Leu Thr Gly Tyr Phe
    210                 215                 220
Val Leu Ser Val Gln Val Met Leu Met Phe Pro Ile Ile Ile His Glu
225                 230                 235                 240
Ile Thr Gly Thr Pro Thr Ala Val Lys Trp Met Tyr Ala Ile Glu Thr
                245                 250                 255
Ala Ile Ser Leu Thr Leu Leu Tyr Pro Ile Ala Arg Trp Ser Glu Lys
            260                 265                 270
His Phe Arg Leu Glu Gln Arg Leu Met Ala Gly Leu Phe Leu Met Ser
        275                 280                 285
Ile Cys Met Phe Pro Ile Gly Trp Val Asn Gln Leu His Thr Leu Phe
    290                 295                 300
Gly Leu Leu Cys Leu Phe Tyr Leu Gly Leu Val Thr Ala Asp Pro Ala
305                 310                 315                 320
Arg Glu Thr Leu Ser Ala Ser Leu Ser Asp Pro Arg Ala Arg Gly Ser
                325                 330                 335
Tyr Met Gly Phe Ser Arg Leu Gly Leu Ala Leu Gly Gly Ala Ile Gly
            340                 345                 350
Tyr Thr Gly Gly Gly Trp Leu Tyr Asp Thr Gly Arg Asp Leu Asn Met
        355                 360                 365
Pro Gln Leu Pro Trp Ile Leu Leu Gly Leu Ser Gly Leu Ile Thr Ile
    370                 375                 380
Tyr Ala Leu His Arg Gln Phe Asn Gln Lys Lys Ile Asp Pro Val Met
385                 390                 395                 400
Leu Gly Arg His
```

<210> 33
<211> 191
<212> PRT
<213> Xenorhabdus bovienii

<400> 33

```
Lys Gly Ala Asn Met Lys Arg Phe Phe Leu Gly Ala Ala Leu Val Leu
 1               5                  10                  15
Val Gly Leu Val Ser Gly Cys Asp Gln Phe Lys Asp Phe Ser Ile Asn
            20                  25                  30
Glu Gly Leu Met Asn Asp Tyr Leu Leu Lys Lys Val His Tyr Gln Lys
        35                  40                  45
Lys Ile Ser Ile Pro Gly Ile Ala Asn Ala Asn Ile Thr Leu Gly Asp
```

```
    50                  55                  60
Leu Ser Ser Gln Ile Gly Arg Gln Asp Pro Glu Lys Ile Glu Leu Ser
65                  70                  75                  80
Thr Gln Ala Lys Val Gln Leu Ala Thr Leu Leu Gly Thr Ile Gln Ala
                85                  90                  95
Asp Met Lys Leu Thr Ile Lys Ala Lys Pro Val Phe Asp Ala Glu Lys
            100                 105                 110
Gly Ala Ile Phe Val Lys Gly Leu Glu Ile Val Asp Tyr Gln Thr Thr
        115                 120                 125
Pro Glu Lys Ala Ala Ala Pro Val Lys Ala Leu Ile Pro Tyr Leu Asn
    130                 135                 140
Thr Ser Leu Ser Glu Phe Phe Asp Thr His Pro Val Tyr Val Leu Asn
145                 150                 155                 160
Pro Glu Lys Ser Lys Ala Glu Ala Ala Ala Ser Gln Phe Ala Lys Arg
                165                 170                 175
Leu Glu Ile Lys Pro Gly Lys Leu Val Ile Gly Leu Thr Asp Lys
            180                 185                 190

<210> 34
<211> 205
<212> PRT
<213> Xenorhabdus bovienii

<400> 34
Gln Val Ala Leu Gln His Gly Arg Arg Leu Gly Thr Ile Thr Leu Phe
 1               5                  10                  15
Asp Asn Leu Leu Gly Leu Asn Gln Val Met Asn Glu Phe Ser Ile Val
            20                  25                  30
Cys Arg Ile Leu Gly Thr Leu Phe Asn Arg Ala Pro Gln Asp Pro Val
        35                  40                  45
Leu Gln Pro Leu Ile Thr Met Ile Ala Glu Gly Lys Leu Lys Gln Ala
    50                  55                  60
Trp Pro Leu Glu Gln Asp Glu Trp Leu Asp Arg Leu Gln Gln Asn Ser
65                  70                  75                  80
Glu Leu Ser Val Met Ala Ala Asp Tyr His Ala Leu Phe Thr Gly Glu
                85                  90                  95
Ser Ala Ser Val Ala Val Cys Arg Ser Asp Tyr Thr Asp Gly Glu Glu
            100                 105                 110
Ser Glu Val Arg Gln Phe Leu Thr Glu Arg Gly Met Pro Leu Ser Asp
        115                 120                 125
Thr Pro Ala Asp Gln Phe Gly Ser Leu Leu Leu Ala Val Ser Trp Leu
    130                 135                 140
Glu Asp Gln Ala Ala Glu Asp Glu Ile Gln Ala Gln Ile Thr Leu Phe
145                 150                 155                 160
Asp Glu Tyr Leu Leu Pro Trp Cys Gly Gln Phe Leu Gly Lys Val Glu
                165                 170                 175
Ala His Ala Thr Ser Gly Phe Tyr Arg Thr Leu Ala Ile Val Thr Arg
            180                 185                 190
Glu Ala Leu Gln Ala Leu Arg Asp Glu Leu Glu Ser Glu
        195                 200                 205

<210> 35
<211> 315
<212> PRT
<213> Xenorhabdus bovienii

<400> 35
```

```
Asp Cys Met Asn Ile Ile Phe Phe His Pro Ser Phe Asn Thr Asp Glu
 1               5                  10                  15
Trp Ile Gln Gly Ile Gln Ala Arg Leu Pro Asp Ala Lys Val Arg Gln
            20                  25                  30
Trp Val Ser Gly Asp Gln Glu Pro Ala Asp Tyr Ala Leu Val Trp Gln
        35                  40                  45
Pro Pro Tyr Glu Met Leu Ala Asn Arg Gln Gly Leu Lys Gly Ile Phe
    50                  55                  60
Ala Leu Gly Ala Gly Val Asp Ala Ile Phe Lys Gln Glu Ser Lys Asn
65                  70                  75                  80
Pro Gly Thr Leu Leu Ala Asp Val Pro Leu Ile Arg Leu Glu Asp Thr
                85                  90                  95
Gly Met Gly Arg Gln Met Gln Glu Tyr Ala Ile Thr Ser Val Leu His
            100                 105                 110
Tyr Phe Arg Arg Met Asp Glu Tyr Lys Arg Tyr Gln Glu Gln Arg Leu
        115                 120                 125
Trp Asn Pro Ile Ala Pro His Asn Arg Lys Glu Phe Val Ile Gly Val
    130                 135                 140
Leu Gly Ala Gly Ile Leu Gly Arg Ser Val Ile Gly Lys Leu Met Glu
145                 150                 155                 160
Phe Asp Phe Asn Val Arg Cys Trp Ser Arg Thr Ser Lys Gln Leu Asp
                165                 170                 175
Ser Val Glu Ser Phe Tyr Gly Lys Glu Gln Leu Gly Asp Phe Leu Ser
            180                 185                 190
Gly Cys Lys Val Leu Ile Asn Leu Leu Pro Asp Thr Pro Asp Thr Arg
        195                 200                 205
Gly Ile Leu Asn Leu Ser Leu Phe Ser Gln Leu Lys Ser Gly Ser Tyr
    210                 215                 220
Val Ile Asn Leu Ala Arg Gly Ala Gln Leu Val Glu Gln Asp Leu Leu
225                 230                 235                 240
Val Ala Ile Asp Lys Gly Tyr Ile Ala Gly Ala Thr Leu Asp Val Phe
                245                 250                 255
Ala Glu Glu Pro Leu Ser Asn Met His Pro Phe Trp Thr His Pro Arg
            260                 265                 270
Ile Asn Val Thr Pro His Ile Ala Ala Asn Thr Ile Pro Glu Ala Ala
        275                 280                 285
Met Asp Val Ile Cys Glu Asn Ile Arg Arg Met Val Gln Gly Glu Met
    290                 295                 300
Pro Thr Gly Leu Val Asp Arg Val Arg Gly Tyr
305                 310                 315

<210> 36
<211> 132
<212> PRT
<213> Xenorhabdus bovienii

<400> 36
Lys Thr Ser Gln Gly Phe Thr Ser Thr Thr Cys Ser Asn Gly Asn Val
 1               5                  10                  15
Leu Lys Ile Cys Gly Leu Ile Thr Pro Cys Ser Ser Leu Ile Gln Arg
            20                  25                  30
Thr Tyr Pro Asn Asn Met Thr Ile Gly Ile Phe Ser Lys Glu Ser Thr
        35                  40                  45
Ala Lys Asn Phe Gly Met Gly Phe Leu Tyr Tyr Phe Asp Leu Arg Val
    50                  55                  60
Leu Ser Pro Phe Phe Lys Ala Pro Ile Asn Ile Phe Thr Gly Trp Gln
65                  70                  75                  80
```

```
His Asn Thr Asn Phe Arg Lys Ser Arg Asn Ser Thr Ile Arg Leu Cys
                85                  90                  95
Ser Ser Thr Pro Asn Ser Lys Gln Tyr Phe Thr Thr Ser Arg Lys Cys
            100                 105                 110
His Ile Thr Gly Ala Gly Lys Tyr Arg Phe Ser Ile Glu Asn Cys Phe
        115                 120                 125
Ile Lys Ser Gly
    130
```

<210> 37
<211> 289
<212> PRT
<213> Xenorhabdus bovienii

<400> 37

```
Tyr Ser Ala Gly Cys Ser Thr Val Leu Lys Ser Ser Leu Asn Leu Gln
 1               5                  10                  15
Cys Asp Thr Phe Asn Cys Glu Ser Phe Val Met Leu Thr Leu Asn Phe
            20                  25                  30
Ser Thr Ser Val Asn Ala Lys Pro Ser His Ile Trp Ala His Tyr Val
        35                  40                  45
Asp Phe Asp Leu Arg Lys Lys Trp Glu Val Asp Leu Glu Tyr Phe Gln
    50                  55                  60
Phe Glu Gly Glu Val Lys Thr Gly Gln Tyr Gly Arg Met Ile Leu Ser
65                  70                  75                  80
Gly Met Pro Glu Ile Arg Phe Tyr Leu Ser Asn Ile Glu Val Asn Lys
                85                  90                  95
Glu Phe Thr Asp Gln Val Asn Leu Pro Gln Met Gly Ile Leu Thr Phe
            100                 105                 110
Arg His Gln Ile Ile Thr Asp Glu Asn Asn Met Ala Cys Arg Val Gln
        115                 120                 125
Val Thr Val Ser Phe Glu Pro Asp Ala Asn Ile Pro Ala Val Gln Ala
    130                 135                 140
Glu Ser Phe Phe Lys Gln Gly Thr Gln Asp Leu Val Glu Ser Val Leu
145                 150                 155                 160
Arg Leu Lys Ser Val Val Glu Thr Val Ser Pro Lys Pro Asn Leu Gln
                165                 170                 175
Leu Val Tyr Val Ser Asp Ile Glu Ser Ser Thr Ala Phe Tyr Lys Thr
            180                 185                 190
Ile Phe Asn Ala Glu Pro Ile Phe Ala Ser Ser Arg Tyr Val Ala Phe
        195                 200                 205
Pro Ala Gly Gly Glu Val Leu Phe Ala Ile Trp Ser Gly Gly Ala Lys
    210                 215                 220
Pro Asp Arg Ala Ile Pro Arg Phe Ser Glu Ile Gly Ile Met Leu Pro
225                 230                 235                 240
Ser Gly Lys Asp Val Asp Arg Cys Phe Glu Glu Trp Arg Lys Asn Pro
                245                 250                 255
Glu Ile Lys Ile Val Gln Glu Pro His Thr Glu Val Phe Gly Arg Thr
            260                 265                 270
Phe Leu Ala Glu Asp Pro Asp Gly His Ile Ile Arg Val Cys Pro Leu
        275                 280                 285
Asp
```

<210> 38
<211> 270
<212> PRT

<213> Xenorhabdus bovienii

<400> 38
Lys Gly Asn Gln Ile Thr Met Ile Leu Tyr Lys Gly Ser Lys Asn Tyr
1 5 10 15
Leu Phe Asn Gln Leu Asn Tyr Asp Ser Cys Val Leu Leu Glu Val Asp
20 25 30
Glu Ser Val Asn Leu Asn Gly Trp Asp Glu Leu Ser Arg Ala Gln Arg
35 40 45
Leu Leu Phe Leu Met Glu Ile Leu Arg Arg Tyr His Phe Pro Val Gln
50 55 60
Gly Lys Val Leu Ala Gln Lys Leu Asn Ile Ser Leu Arg Thr Leu Tyr
65 70 75 80
Arg Asp Ile Ala Ser Leu Gln Ala Gln Gly Ala Ile Ile Glu Gly Glu
85 90 95
Pro Gly Ile Gly Tyr Val Leu Arg Pro Gly Phe Val Leu Pro Pro Leu
100 105 110
Met Phe Thr Gln Asn Glu Ile Glu Ala Leu Ala Leu Gly Ala Asn Trp
115 120 125
Val Ala Lys Arg Ala Asp Pro Gln Leu Lys Glu Ser Ala Asn Asn Ala
130 135 140
Ile Ser Lys Ile Ala Ala Val Ile Pro Ala Glu Leu Lys Gln Met Leu
145 150 155 160
Glu Ala Ser Ser Leu Leu Ile Gly Pro Ala Ala Thr Ala Val Gln Pro
165 170 175
Val Val Glu Ile Gln Gln Ile Arg Gln Ala Ile Asn Thr Arg His Lys
180 185 190
Ile Thr Leu Ala Tyr Leu Asp Ile Lys Asp Ile Pro Ser Glu Arg Thr
195 200 205
Ile Trp Pro Phe Ala Leu Gly Tyr Phe Glu Asn Ile Ser Ile Val Ile
210 215 220
Gly Trp Cys Glu Leu Arg Glu Glu Phe Arg His Phe Arg Ser Asp Arg
225 230 235 240
Ile Met Arg Leu Lys Ile Glu Asn Gln Cys Tyr Pro Arg Ser Arg Gln
245 250 255
Val Leu Leu Lys Glu Trp Arg Ala Met Glu Lys Ile Ser Arg
260 265 270

<210> 39
<211> 209
<212> PRT
<213> Xenorhabdus bovienii

<400> 39
Arg Lys Met Thr Ile Tyr Asp Leu Lys Pro Arg Phe Gln Asn Leu Leu
1 5 10 15
Arg Pro Ile Val Ile Tyr Leu Tyr Lys Gln Gly Ile Thr Ala Asn Gln
20 25 30
Val Thr Leu Thr Ala Leu Phe Leu Ser Ile Phe Ala Gly Ser Leu Leu
35 40 45
Ser Leu Phe Pro Ser Pro His Leu Tyr Trp Leu Leu Pro Val Phe Leu
50 55 60
Phe Ile Arg Met Ala Leu Asn Ala Ile Asp Gly Met Leu Ala Arg Glu
65 70 75 80
His Asn Gln Lys Ser His Leu Gly Ala Ile Tyr Asn Glu Leu Gly Asp
85 90 95
Val Ile Ser Asp Val Ala Leu Tyr Leu Pro Phe Cys Leu Leu Pro Asp

```
            100                 105                 110
Val Asn Ser Leu Ser Leu Leu Ile Ile Leu Phe Leu Thr Ile Leu Thr
        115                 120                 125
Glu Phe Ile Gly Val Leu Ala Gln Thr Ile Gly Ala Ser Arg Arg Tyr
    130                 135                 140
Asp Gly Pro Ile Gly Lys Ser Asp Arg Ala Phe Ile Phe Gly Ala Tyr
145                 150                 155                 160
Gly Leu Ile Ile Ala Ile Phe Pro Leu Ala Leu Gly Trp Ser Ile Ser
                165                 170                 175
Leu Phe Ala Phe Met Ile Ile Leu Leu Leu Val Thr Cys Tyr Gln Arg
            180                 185                 190
Val Val Lys Ala Leu Arg Glu Ile Arg Leu Ala Glu Gln Ser His Ser
        195                 200                 205
Lys
```

<210> 40
<211> 592
<212> PRT
<213> Xenorhabdus bovienii

<400> 40

```
Gly Val Asn Met Thr Pro Gln Leu Asp Gln Arg Ile Ala Glu Glu His
 1               5                  10                  15
Tyr Phe Thr Thr Ser Asp Asn Ala Ser Leu Phe Tyr Arg Tyr Trp Pro
            20                  25                  30
Gln Gln Gln Ala Asn Pro Asp Arg Ala Ile Ile Ile Phe His Arg Gly
        35                  40                  45
His Glu His Ser Gly Arg Ile Gln His Val Val Asp Gly Leu Asp Leu
    50                  55                  60
Pro Asp Val Pro Met Phe Ala Trp Asp Ala Arg Gly His Gly Lys Thr
65                  70                  75                  80
Glu Gly Pro Arg Gly Tyr Ser Pro Ser Met Gly Thr Ser Ile Arg Asp
                85                  90                  95
Val Asp Glu Phe Val Arg Phe Ile Ala Thr Gln Tyr Gly Ile Ala Met
            100                 105                 110
Glu Asn Ile Val Val Ile Gly Gln Ser Val Gly Ala Val Leu Val Ser
        115                 120                 125
Ala Trp Val His Asp Tyr Ala Pro Lys Ile Arg Ala Met Ile Leu Ala
    130                 135                 140
Ala Pro Ala Phe Asp Ile Lys Leu Tyr Ile Pro Phe Ala Thr Gln Gly
145                 150                 155                 160
Leu Gln Leu Met Gln Lys Ala Arg Gly Ile Phe Phe Val Asn Ser Tyr
                165                 170                 175
Val Lys Ala Arg Tyr Leu Thr His Asp Glu Thr Arg Ile Ala Ser Tyr
            180                 185                 190
Asn Ser Asp Pro Leu Ile Thr Arg Glu Ile Ala Val Asn Ile Leu Leu
        195                 200                 205
Asp Leu Tyr Gln Thr Ala Glu Arg Val Val Lys Asp Ala Ala Ala Ile
    210                 215                 220
Thr Leu Pro Thr Leu Leu Phe Ile Ser Gly Ser Asp Tyr Val Val Asn
225                 230                 235                 240
Lys Lys Pro Gln His Gln Phe Tyr Gln Gln Leu Asn Thr Pro Ile Lys
                245                 250                 255
Glu Lys His Val Met Asp Gly Phe Tyr His Asp Thr Leu Gly Glu Lys
            260                 265                 270
Asp Arg His Leu Val Phe Asp Lys Ile Arg Val Phe Ile Glu Arg Ile
```

```
        275                 280                 285
Phe Ala Leu Pro Arg Tyr Gln His Asp Tyr Ser Gln Glu Asp Thr Trp
    290                 295                 300
Ser His Ser Ala Asp Glu Phe Arg Thr Leu Ser Thr Ser Leu Pro Cys
305                 310                 315                 320
Leu Cys Pro Lys Lys Leu Ser Tyr Gln Leu Met Arg Lys Val Met Ser
                325                 330                 335
Thr His Trp Gly Arg Thr Ser Glu Gly Val Cys Ile Gly Leu Lys Thr
            340                 345                 350
Gly Phe Asp Ser Gly Ser Thr Leu Asp Tyr Val Tyr Arg Asn Gln Pro
        355                 360                 365
Gln Gly Lys Gly Ile Leu Gly Arg Ile Leu Asp Lys His Tyr Leu Asn
    370                 375                 380
Ser Ile Gly Trp Arg Gly Ile Arg Gln Arg Lys Ile His Ile Glu Met
385                 390                 395                 400
Leu Ile Arg His Ala Ile Arg Ser Leu Arg Glu Gln Asn Met Pro Val
                405                 410                 415
His Met Val Asp Ile Ala Ala Gly His Gly Arg Tyr Ile Leu Asp Ala
            420                 425                 430
Ile Asn Asp Phe Ser Lys Val Asp Ser Ile Leu Leu Arg Asp Tyr Ser
        435                 440                 445
Glu Ile Asn Val Asn Gln Gly Gln Ala Tyr Ile Glu Glu Arg Asp Leu
    450                 455                 460
Thr Asp Lys Ile Arg Phe Ile Ile Gly Asp Ala Phe Asn Ala Glu Ser
465                 470                 475                 480
Ile Ser Ser Ile Thr Pro Ala Pro Thr Leu Gly Ile Val Ser Gly Leu
                485                 490                 495
Tyr Glu Leu Phe Pro Asp Asn Asn Leu Leu Arg Asn Ser Leu Arg Gly
            500                 505                 510
Phe Ala Asp Val Met Thr Glu Asn Gly Tyr Leu Val Tyr Thr Gly Gln
        515                 520                 525
Pro Trp His Pro Gln Ile Glu Val Ile Ala Arg Val Leu Ser Ser His
    530                 535                 540
Arg Asp Ser Gln Pro Trp Ile Met Arg Arg Arg Thr Gln Gly Glu Met
545                 550                 555                 560
Asp Ala Leu Val Glu Ala Ala Gly Phe Glu Lys Leu Tyr Gln Leu Thr
                565                 570                 575
Asp Asn Trp Gly Ile Phe Thr Val Ser Ile Ala Lys Arg Val His Arg
            580                 585                 590
```

<210> 41
<211> 121
<212> PRT
<213> Xenorhabdus bovienii

<400> 41

```
His His Asn Ser Ile Asn Val Leu Leu Lys Asn Ile Ile Ser Pro His
 1               5                  10                  15
Gln Ile Met Leu Leu Cys Phe Thr Val Thr Gly His Asn Asn Arg Pro
            20                  25                  30
Ile Gln Thr Glu Arg Ser Leu Phe Phe Thr Val Val Met Ser Thr Gln
        35                  40                  45
Asp Val Ser Ser Met Ser Leu Thr Asp Ser Ile Cys Leu Met Phe Leu
    50                  55                  60
Cys Ser Arg Gly Met Pro Val Asp Thr Val Arg Gln Lys Gly Arg Ala
65                  70                  75                  80
Val Thr Ala His Pro Trp Glu Arg Arg Phe Val Met Leu Met Asn Leu
```

```
                85                  90                  95
Ser Asp Leu Leu Pro Leu Ser Thr Ala Ser Pro Trp Lys Ile Ser Trp
            100                 105                 110
Leu Ser Ala Arg Val Ser Glu Arg Tyr
        115                 120
```

<210> 42
<211> 444
<212> PRT
<213> Xenorhabdus bovienii

<400> 42

```
Ile Asn Lys Tyr Lys Met Glu His His Met His Ser Ser Leu Asp Ser
 1               5                  10                  15
Arg Arg Arg Leu Trp Leu Thr Gly Val Ile Trp Leu Leu Phe Leu Ala
            20                  25                  30
Pro Phe Phe Phe Leu Thr Tyr Gly Gln Val Asn Gln Phe Thr Ala Gln
        35                  40                  45
Arg Ser Asp Val Gly Thr Val Met Phe Gly Trp Glu His Asn Ile Pro
    50                  55                  60
Phe Trp Ser Trp Ser Ile Ile Pro Tyr Trp Ser Ile Asp Leu Phe Tyr
65                  70                  75                  80
Gly Ile Ser Leu Phe Ile Cys Thr His Arg Arg Glu Gln Trp Leu His
                85                  90                  95
Gly Trp Arg Leu Met Thr Ala Ser Leu Ile Ala Cys Val Gly Phe Leu
            100                 105                 110
Leu Phe Pro Leu Lys Phe Ser Phe Ser Arg Pro Thr Thr Glu Gly Leu
        115                 120                 125
Phe Gly Trp Leu Phe Asn Gln Leu Glu Leu Phe Asp Leu Pro Tyr Asn
    130                 135                 140
Gln Ala Pro Ser Leu His Ile Ile Leu Leu Trp Leu Leu Trp Leu Arg
145                 150                 155                 160
Tyr Ser Ala Tyr Val Ser Gly Tyr Trp Arg Gly Leu Leu His Ile Trp
                165                 170                 175
Ser Val Leu Ile Ala Leu Ser Val Leu Thr Thr Trp Gln His His Phe
            180                 185                 190
Ile Asp Val Leu Thr Gly Phe Ala Val Gly Val Ile Leu Ser Tyr Leu
        195                 200                 205
Leu Pro Val Ser Tyr Arg Trp Arg Trp Gln Pro Asn Gln Asp Arg Tyr
    210                 215                 220
Ala Arg Lys Leu Phe Gly Tyr Tyr Leu Thr Gly Ser Ala Leu Phe Ala
225                 230                 235                 240
Leu Ile Ala Ser Leu Leu Gly Gly Ser Phe Trp Ile Leu Leu Trp Pro
                245                 250                 255
Ala Val Ser Leu Leu Met Ile Ala Leu Gly Tyr Ala Gly Leu Gly Ser
            260                 265                 270
Ser Val Phe Gln Lys Gln Pro Asp Gly Arg Met Ser Leu Ser Ala Arg
        275                 280                 285
Trp Leu Leu Ala Pro Tyr Gln Leu Gly Ala Trp Leu Ser Tyr Leu Trp
    290                 295                 300
Phe Arg Arg Lys Ser Ala Pro Phe Asn His Ile Thr Glu Gly Ile Ile
305                 310                 315                 320
Leu Gly Ser Leu Pro Cys Gln Pro Val Thr Ala Val Ser Val Leu Asp
                325                 330                 335
Ile Thr Ala Glu Trp His Arg Arg Ser Asp Ala Arg Thr Val Asn Tyr
            340                 345                 350
Val Cys Gln Pro Gln Ile Asp Leu Leu Pro Leu Ala Pro Glu Ala Leu
```

```
        355                 360                 365
Gln Ser Ala Val Cys Thr Leu Asp Lys Leu Arg Gln Gln Gly Asp Val
    370                 375                 380
Phe Val His Cys Thr Leu Gly Leu Ser Arg Ser Ala Met Val Val Ala
385                 390                 395                 400
Ala Trp Leu Leu Lys Gln His Pro Glu Tyr Asp Ile Asn Thr Val Val
                405                 410                 415
Ala Ile Leu Arg Lys Ala Arg Pro His Val Thr Phe Arg Gln Thr His
            420                 425                 430
Leu Asp Ala Leu Ser Gln Trp Ala Lys Gly Tyr Leu
        435                 440
```

<210> 43
<211> 174
<212> PRT
<213> Xenorhabdus bovienii

<400> 43

```
Gln Ser Cys Val Lys Pro Asp Arg Met Ser Arg Ser Asp Lys His Ile
 1               5                  10                  15
Trp Met Pro Cys Leu Asn Gly Gln Lys Ala Thr Tyr Asn Gly Glu His
            20                  25                  30
Asn Met Gln Pro Glu Asn Leu Ile Ser Lys Val Ile Ile Ala Thr Leu
        35                  40                  45
Lys Ser Trp Arg Phe Ile Ser Thr Leu Ser Ala Phe Ser Ile Leu Ile
    50                  55                  60
Ala Thr Ala Met Leu Ile Ala Val Phe Asn Thr Thr Ala Leu Asn Asn
65                  70                  75                  80
Ile Ala Leu Tyr Ala Val Leu Leu Phe Thr Thr Leu Tyr Cys Gln Tyr
                85                  90                  95
Tyr Cys Trp Arg Thr Trp Leu Asp Cys His Tyr Phe Gln Ile Leu Asn
            100                 105                 110
Ser Ser Pro Glu Lys Ser Ala Glu Phe Asp Gln Thr Leu Leu Leu Ile
        115                 120                 125
Phe Asn Lys Leu Pro Gln Ser Arg Thr Gln Asn Asp Arg Phe Asn Gly
    130                 135                 140
Ala Ile Lys Leu Leu Lys Lys Ala Thr Ile Gly Leu Ile Leu Gln Trp
145                 150                 155                 160
Ile Leu Phe Phe Leu Phe Leu Leu Thr Leu Lys Tyr Ser Ala
                165                 170
```

<210> 44
<211> 466
<212> PRT
<213> Xenorhabdus bovienii

<400> 44

```
Met Asn Thr Arg Lys Ile Asn Gly Ile Arg Pro Phe Ser Ala Phe Ile
 1               5                  10                  15
Asp Ser Cys Leu Lys Glu Ser Tyr Ser Phe Pro Arg Phe Ile Arg Asp
            20                  25                  30
Ile Ile Ala Gly Ile Thr Val Gly Val Ile Ala Ile Pro Leu Ala Met
        35                  40                  45
Ala Leu Ala Ile Gly Ser Gly Val Ala Pro Gln Tyr Gly Leu Tyr Thr
    50                  55                  60
Ala Ala Ile Ala Gly Ile Val Ile Ala Met Thr Gly Gly Ser Arg Tyr
65                  70                  75                  80
```

```
Ser Val Ser Gly Pro Thr Ala Ala Phe Val Val Ile Leu Tyr Pro Val
                85                  90                  95
Ser Gln Gln Phe Gly Leu Ser Gly Leu Leu Ile Ala Thr Leu Met Ser
            100                 105                 110
Gly Val Ile Leu Ile Val Met Gly Leu Ala Arg Phe Gly Arg Leu Ile
        115                 120                 125
Glu Tyr Ile Pro Met Ser Val Thr Leu Gly Phe Thr Ser Gly Ile Ala
    130                 135                 140
Ile Thr Ile Ala Thr Met Gln Val Gln Asn Phe Phe Gly Leu Lys Leu
145                 150                 155                 160
Ala His Ile Pro Glu Asn Tyr Ile Asp Lys Val Val Ala Leu Tyr Gln
                165                 170                 175
Ala Leu Pro Ser Leu Gln Leu Ser Asp Thr Leu Ile Gly Leu Thr Thr
            180                 185                 190
Leu Leu Val Leu Ile Phe Trp Pro Lys Leu Gly Val Lys Leu Pro Gly
        195                 200                 205
His Leu Pro Ala Leu Ile Ala Gly Thr Ala Val Met Gly Ala Met His
    210                 215                 220
Leu Leu Asn His Asp Val Ala Thr Ile Gly Ser Ser Phe Ser Tyr Thr
225                 230                 235                 240
Leu Ala Asp Gly Thr Gln Gly Gln Gly Ile Pro Pro Ile Leu Pro Gln
                245                 250                 255
Phe Val Leu Pro Trp Asn Leu Pro Asp Thr His Ser Leu Asp Ile Ser
            260                 265                 270
Trp Asn Thr Val Ser Ala Leu Leu Pro Ala Ala Phe Ser Met Ala Met
        275                 280                 285
Leu Gly Ala Ile Glu Ser Leu Leu Cys Ala Val Ile Leu Asp Gly Met
    290                 295                 300
Thr Gly Lys Lys His His Ser Asn Gly Glu Leu Leu Gly Gln Gly Leu
305                 310                 315                 320
Gly Asn Ile Ala Ala Pro Phe Phe Gly Gly Ile Thr Ala Thr Ala Ala
                325                 330                 335
Ile Ala Arg Ser Ala Ala Asn Val Arg Ala Gly Ala Thr Ser Pro Ile
            340                 345                 350
Ala Ala Val Val His Ser Leu Leu Val Leu Leu Thr Leu Leu Val Leu
        355                 360                 365
Ala Pro Met Leu Ser Tyr Leu Pro Leu Ala Ala Met Ser Ala Ile Leu
    370                 375                 380
Leu Ile Val Ala Trp Asn Met Ser Glu Ala His Lys Val Val Asp Leu
385                 390                 395                 400
Ile Arg His Ala Pro Lys Asp Asp Ile Ile Val Met Leu Leu Cys Leu
                405                 410                 415
Ser Leu Thr Val Leu Phe Asp Met Val Arg Arg Asp His Tyr Arg His
            420                 425                 430
Cys Ala Gly Ile Thr Pro Val Tyr Ala Gln Asn Cys Gln Tyr Asp Ser
        435                 440                 445
Asn Gln His Val Ile Phe Asn Lys Arg Gly Glu Arg Val Ile Gly Arg
    450                 455                 460
Thr Asn
465

<210> 45
<211> 125
<212> PRT
<213> Xenorhabdus bovienii

<400> 45
```

```
Glu Ser Ile Gly Ala Lys Thr Ser Asn Val Asn Asn Thr Ser Arg Glu
 1               5                  10                  15
Cys Thr Thr Ala Ala Ile Gly Glu Val Ala Pro Ala Arg Thr Leu Ala
            20                  25                  30
Ala Glu Arg Ala Ile Ala Ala Val Ala Val Met Pro Pro Lys Lys Gly
        35                  40                  45
Ala Ala Ile Leu Pro Asn Pro Trp Pro Ser Ser Ser Pro Leu Glu Trp
    50                  55                  60
Cys Phe Phe Pro Val Ile Pro Ser Arg Ile Thr Ala His Ser Asn Asp
65                  70                  75                  80
Ser Ile Ala Pro Ser Met Ala Ile Glu Asn Ala Ala Gly Ser Asn Ala
                85                  90                  95
Asp Thr Val Phe Gln Leu Ile Ser Arg Glu Cys Val Ser Gly Lys Phe
            100                 105                 110
His Gly Arg Thr Asn Trp Gly Arg Met Gly Gly Met Pro
        115                 120                 125
```

<210> 46
<211> 161
<212> PRT
<213> Xenorhabdus bovienii

```
<400> 46
Leu Ser Tyr Ser Ile Trp Ser Val Ala Ile Thr Ile Gly Ile Val Leu
 1               5                  10                  15
Ala Ser Leu Leu Phe Met Arg Lys Ile Ala Asn Met Thr Arg Ile Ser
            20                  25                  30
Thr Ser Ser Leu Thr Ser Ala Glu Lys Gly Leu Leu Val Val Arg Ile
        35                  40                  45
Asn Gly Pro Leu Phe Phe Ala Ala Ala Glu Arg Ile Phe Ala Glu Leu
    50                  55                  60
Arg Glu Lys Ser Ala Asp Tyr Gln Thr Ile Ile Met Gln Trp Asp Ala
65                  70                  75                  80
Val Pro Val Leu Asp Ala Gly Gly Leu His Ala Phe Gln Gly Phe Val
                85                  90                  95
Arg Glu Leu Gly Lys Glu Lys His Ile Val Val Cys Asp Ile Pro Phe
            100                 105                 110
Gln Pro Leu Lys Thr Leu Ala Arg Ala Lys Val Met Pro Ile Glu Gly
        115                 120                 125
Glu Leu Ser Phe Tyr Ala Thr Leu Pro Lys Ala Leu Lys Glu Met Ala
    130                 135                 140
Val Asp Tyr Thr Pro Glu Val Cys Ala Ser Ser Glu Lys Ile Gln Gly
145                 150                 155                 160
Gln
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<210> 47
<211> 173
<212> PRT
<213> Xenorhabdus bovienii

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<400> 47
Cys Met Ser Asp Val Glu Asn Asp Arg Arg Thr Leu Gly Ser Leu Leu
 1               5                  10                  15
His Asp Thr Glu Ala Gln His Val Asn His Gln Ile Val Ile Thr Lys
            20                  25                  30
Val Ala Ala Thr Val Thr Gln Asp His Leu Val Ile Ala Ala Phe Phe
```

```
        35                  40                  45
Glu Phe Phe Asn Asn Ile Ala His Leu Pro Arg Ala Asn Lys Leu Trp
    50                  55                  60
Phe Phe Asn Ile Asn His Ser Thr Gly Phe Arg His Arg Phe Asn Gln
65                  70                  75                  80
Ile Gly Leu Ala Gly Lys Glu Gly Trp Lys Leu Asn His Ile His His
                85                  90                  95
Ile Arg Asp Trp Leu Ser Leu Cys Arg Leu Met His Val Ser Asp Asn
            100                 105                 110
Phe His Ala Glu Gly Leu Phe Gln Phe Leu Lys Asp Phe His Pro Leu
        115                 120                 125
Phe Gln Pro Trp Pro Thr Ile Arg Ala Asp Arg Arg Thr Val Ser Leu
    130                 135                 140
Ile Lys Arg Arg Phe Lys Asn Ile Arg Asn Ala Gln Phe Leu Cys His
145                 150                 155                 160
Gly Asp Ile Val Leu Thr Asn Pro His Gly Gln Ile Pro
                165                 170

<210> 48
<211> 308
<212> PRT
<213> Xenorhabdus bovienii

<400> 48
Leu Ser Cys Ile Arg Phe Ile Phe Leu Leu Ile Gln Gln Ile Tyr Leu
1               5                   10                  15
Pro Leu Thr Arg Glu Gly Ile Ser Met Gln Gln Lys Val Val Asn Ile
            20                  25                  30
Gly Asp Ile Lys Val Ala Asn Asp Leu Pro Phe Val Leu Phe Gly Gly
        35                  40                  45
Met Asn Val Leu Glu Ser Arg Asp Leu Ala Met Arg Ile Cys Glu His
    50                  55                  60
Tyr Val Thr Val Thr Gln Lys Leu Gly Ile Pro Tyr Val Phe Lys Ala
65                  70                  75                  80
Ser Phe Asp Lys Ala Asn Arg Ser Ser Ile Arg Ser Tyr Arg Gly Pro
                85                  90                  95
Gly Leu Glu Glu Gly Met Lys Ile Phe Gln Glu Leu Lys Gln Thr Phe
            100                 105                 110
Gly Val Lys Ile Ile Thr Asp Val His Glu Pro Ala Gln Ala Gln Pro
        115                 120                 125
Val Ala Asp Val Val Asp Val Ile Gln Leu Pro Ala Phe Leu Ala Arg
    130                 135                 140
Gln Thr Asp Leu Val Glu Ala Met Ala Lys Thr Gly Ala Val Ile Asn
145                 150                 155                 160
Val Lys Lys Pro Gln Phe Val Ser Pro Gly Gln Met Gly Asn Ile Val
                165                 170                 175
Glu Lys Phe Lys Glu Gly Gly Asn Asp Gln Val Ile Leu Cys Asp Arg
            180                 185                 190
Gly Ser Asn Phe Gly Tyr Asp Asn Leu Val Val Asp Met Leu Gly Phe
        195                 200                 205
Gly Val Met Gln Gln Ala Thr Gln Gly Ala Pro Val Ile Phe Asp Val
    210                 215                 220
Thr His Ala Leu Gln Cys Arg Asp Pro Leu Gly Ala Ala Ser Gly Gly
225                 230                 235                 240
Arg Arg Ala Gln Val Ala Glu Leu Ala Arg Ala Gly Met Ala Val Gly
                245                 250                 255
Ile Ala Gly Leu Phe Leu Glu Ala His Pro Asp Pro Glu Asn Ala Lys
```

```
            260                 265                 270
Cys Asp Gly Pro Ser Ala Leu Pro Leu Ala Lys Leu Glu Ser Phe Leu
        275                 280                 285
Met Gln Ile Lys Ala Ile Asp Asp Val Val Lys Asn Phe Pro Glu Leu
    290                 295                 300
Asp Thr Ser Lys
305
```

<210> 49
<211> 274
<212> PRT
<213> Xenorhabdus bovienii

<400> 49

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Val Asp Gly Ile Lys Met Lys Pro Ile Val Asn Tyr Glu Phe Asn Asn
 1               5                  10                  15
Thr Pro Leu Ile Asp Gly Ile Ile Leu Val Ser Lys Ile Ile Arg Pro
            20                  25                  30
Asp Phe Pro Gln Thr Leu Val Ser Glu Gln Leu Thr Ala Leu Val Glu
        35                  40                  45
Glu Ala Arg Gln Arg Leu Ser Ser Ile Thr Asp Ser Lys Val Lys Leu
    50                  55                  60
Asp Ser Leu Leu Thr Leu Phe Tyr Arg Glu Trp Lys Phe Gly Gly Ala
65                  70                  75                  80
Asn Gly Val Tyr Cys Leu Ser Asp Thr Leu Trp Leu Asp Arg Leu Leu
                85                  90                  95
His Ser Arg Gln Gly Ser Pro Val Ser Leu Gly Thr Val Phe Thr His
            100                 105                 110
Ile Ala Gln Ala Leu Gly Leu Ser Val Gln Pro Val Ile Phe Pro Ile
        115                 120                 125
Gln Leu Ile Leu Arg Ile Asp Leu Leu Asp Gln Pro Thr Trp Phe Ile
    130                 135                 140
Asn Pro Leu Asn Gly Asp Thr Leu Asn Glu His Thr Leu Asp Val Trp
145                 150                 155                 160
Leu Lys Gly Asn Ile Gly Pro Thr Val Arg Leu Lys Lys Gln Asp Leu
                165                 170                 175
Gln Glu Ala Asp Asn Val Ser Leu Val Arg Lys Ile Thr Asp Thr Ile
            180                 185                 190
Lys Val Ser Leu Met Glu Glu Lys Lys Met Glu Leu Ala Leu Lys Ala
        195                 200                 205
Ser Glu Val Val Leu Thr Phe Asp Pro Asp Asp Pro Tyr Glu Ile Arg
    210                 215                 220
Asp Arg Gly Leu Ile Tyr Ala Gln Leu Asp Cys Asn His Ile Ala Val
225                 230                 235                 240
Ser Asp Leu Ser Tyr Phe Val Glu His Cys Pro Glu Asp Pro Ile Ser
                245                 250                 255
Glu Met Ile Lys Met Gln Ile Asn Thr Ile Glu Gln Arg Leu Ile Val
            260                 265                 270
Leu His
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<210> 50
<211> 316
<212> PRT
<213> Xenorhabdus bovienii

<400> 50

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Ser Asp Arg Arg Gln Thr Gly Tyr Ala Tyr Ser Ala Asp His Tyr Arg
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Ile Ser Gly Arg Ser Thr Val Cys Thr Val Arg Ala Gly Leu Met Asn
            20                  25                  30
Tyr Gln Cys Trp Leu Gln His Ala Ala Thr Gln Leu Ser Glu Ser Asp
        35                  40                  45
Ser Pro Lys Arg Asp Ala Glu Ile Leu Leu Gly Tyr Val Thr Gly Arg
    50                  55                  60
Ser Arg Thr Tyr Leu Ile Ala Phe Asp Glu Thr Leu Ile Ser Ser Glu
65                  70                  75                  80
Glu Leu His Gln Leu Asp Ser Leu Leu Val Arg Arg Ile Gln Gly Glu
                85                  90                  95
Pro Val Ala Tyr Ile Ile Gly Glu Arg Glu Phe Trp Ser Leu Pro Phe
            100                 105                 110
Ala Val Ser Pro Ala Thr Leu Ile Pro Arg Pro Asp Thr Glu Cys Leu
        115                 120                 125
Val Glu Lys Ala Leu Glu Leu Leu Pro Asp Ser Pro Ala Arg Ile Leu
    130                 135                 140
Asp Leu Gly Thr Gly Thr Gly Ala Ile Ala Leu Ala Leu Ala Ser Glu
145                 150                 155                 160
Arg Asn Asp Cys Tyr Val Thr Gly Val Asp Ile Asn Ser Asp Ala Val
                165                 170                 175
Met Leu Ala Gln His Asn Ala Glu Lys Asn Ala Gly Lys Leu Ala Ile
            180                 185                 190
His Asn Val Asn Phe Leu Gln Ser Glu Trp Phe Ala Ala Val Gly Asn
        195                 200                 205
Gln Gln Phe Asp Met Ile Val Ser Asn Pro Pro Tyr Ile Asp Glu Arg
    210                 215                 220
Asp Pro His Leu Gln Glu Gly Asp Ile Arg Phe Glu Pro Ala Thr Ala
225                 230                 235                 240
Leu Ile Ala Ala Gln Asn Gly Met Ala Asp Leu Gln Ala Ile Val Gly
                245                 250                 255
Gln Ala Arg His Phe Leu Ser Pro Asn Gly Trp Leu Leu Leu Glu His
            260                 265                 270
Gly Trp Lys Gln Gly Thr Val Val Arg Asn Leu Phe Leu Glu Lys Gly
        275                 280                 285
Tyr Gln Gln Ile Ala Thr Phe Gln Asp Tyr Gly Gly Asn Glu Arg Ile
    290                 295                 300
Thr Ile Gly Arg Trp Asn Lys Asn Glu Thr His Ser
305                 310                 315
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<210> 51
<211> 289
<212> PRT
<213> Xenorhabdus bovienii

<400> 51

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Val Glu Met Arg Glu Met Ala Gln Glu Glu Leu Lys Glu Ala Lys Ile
 1               5                  10                  15
Arg Asn Glu Glu Leu Glu Gln Gln Leu Gln Leu Leu Leu Leu Pro Lys
            20                  25                  30
Asp Pro Asp Asp Glu Arg Asn Cys Phe Leu Glu Val Arg Ala Gly Thr
        35                  40                  45
Gly Gly Asp Glu Ala Ala Ile Phe Ala Gly Asp Leu Phe Arg Met Tyr
    50                  55                  60
Ser Arg Tyr Ala Glu Ala Arg Arg Trp Arg Val Glu Ile Ile Ser Ala
65                  70                  75                  80
```

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Asn Glu Gly Glu His Gly Gly Tyr Lys Glu Val Ile Ala Lys Val Ser
                85                  90                  95
Gly Asp Gln Val Tyr Gly His Leu Lys Phe Glu Ser Gly Gly His Arg
            100                 105                 110
Val Gln Arg Val Pro Glu Thr Glu Ser Gln Gly Arg Ile His Thr Ser
        115                 120                 125
Ala Cys Thr Val Ala Val Met Pro Glu Ile Pro Glu Ala Glu Leu Pro
    130                 135                 140
Asp Ile Ser Pro Gly Asp Leu Lys Ile Asp Thr Phe Arg Ser Ser Gly
145                 150                 155                 160
Ala Gly Gly Gln His Val Asn Thr Thr Asp Ser Ala Ile Arg Ile Thr
                165                 170                 175
His Leu Pro Thr Gly Ile Val Val Glu Cys Gln Asp Glu Arg Ser Gln
            180                 185                 190
His Lys Asn Lys Ala Lys Ala Met Ser Val Leu Ala Ala Arg Ile Arg
        195                 200                 205
Ala Ala Glu Met Arg Lys Arg Gln Glu Val Glu Ala Ser Glu Arg Arg
    210                 215                 220
Asn Leu Leu Gly Ser Gly Asp Arg Ser Asp Arg Asn Arg Thr Tyr Asn
225                 230                 235                 240
Phe Pro Gln Gly Arg Val Thr Asp His Arg Ile Asn Leu Thr Leu Tyr
                245                 250                 255
Arg Leu Asp Glu Val Ile Glu Gly Lys Leu Asp Met Leu Ile Gln Pro
            260                 265                 270
Ile Ile Ile Glu Tyr Gln Ala Asp Gln Leu Ser Ala Leu Ser Glu Gln
        275                 280                 285
Asp
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<210> 52
<211> 37544
<212> DNA
<213> Xenorhabdus bovienii

<400> 52

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